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DRUG, HOST AND PARASITE INTERRELATIONSHIPS IN THE TREATMENT OF AVIAN MALARIA (*PLASMODIUM CATHEMERIUM* IN CANARIES)

RUSSELL O. HANSON* AND ARTHUR L. TATUM
From the Department of Pharmacology and Toxicology,
University of Wisconsin, Madison, Wisconsin

Successful treatment of man or infected animals involves at least three factors: the parasite, the host, and the therapeutic agent.[1] Clinically as well as experimentally, ordinarily effective therapeutic agents occasionally fail, which fact necessitates the assumption of the failure of adequate response on the part of the host or some change in the infective agent. In such instances the drug or chemical agent may be considered as a constant, whereas the individual host may differ significantly from others of the same species or even of the same immediate ancestry. Furthermore, the infective agent itself may change in virulence or susceptibility to the drug. The treatment of avian malaria is successful in certain species of birds infected by appropriate parasites, while in other avian hosts the same infection may be incurable. In man, malignant tertian (*Plasmodium falciparum*) malaria is relatively successfully controllable by treatment, whereas benign tertian (*Plasmodium vivax*) malaria is, and has been, very resistant in respect to complete eradication. Malarial organisms infective to ducks or fowls can be readily controlled, whereas *Plasmodium cathemerium* and certain other species infective to canaries cause an essentially incurable infection or latency, regardless of type or duration of therapy. This can be interpreted to mean that the canary has an extremely low natural capacity for developing an effective resistance to malaria.

In order to understand better some of the conditions basic to the causes of therapeutic difficulties in the way of treatment, the canary, infected by *P. cathemerium*, was made the subject of study, particularly in respect to the question of when and how an infection becomes firmly established.

In the instance of avian malaria in the canary, such as *P. cathemerium*, for example, neither the mosquito-borne nor the blood-to-blood transfer form of the disease can be cured by any safe means. Gingrich[2] has reported a radical cure with a naphthoquinone derivative, but the dosage required is at the LD_{50} level for the canary. These results we have confirmed. One-half of our group was cured, whereas the other half died from the effects of the toxicity of the drug. Both man and canary develop latent infections; but the latency in the canary, however the infection is established, is practically beyond control, whereas in man the vivax infection

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* Present address: Chicago Medical School, Chicago 12, Ill.

1. Pfeiffer, C. C. and Tatum, A. L. 1935, A new experimental approach to the study of the role of the reticulo-endothelial system in the cure of trypanosomiasis. J. Pharmacol. & Exper. Therap. **53**: 358–376.

2. Gingrich, W., Schoch, E. W., Schwab, M. and Shepherd, C. C. 1947, Radical cure of avian malaria (*P. cathemerium*) with SN 8557, a naphthoquinone derivative. Am. J. Trop. Med. **27**: 147–152.

TABLE 1.—*Results of the intravenous inoculation of fixed numbers of parasites followed immediately by the intravenous administration of 150 μg of pentaquine.*

Number of birds used	Number of parasites inoculated	Infected birds	Non-infected birds
27	5 to 10,000	0	27
30	17,000 to 56,000	13	17
7	100,000 to 1 million	7	0

induced by blood-to-blood transfer (absence of sporozoite phase) is readily subject to successful therapy.

EXPERIMENTAL

In order to determine when and how an infection, such as *P. cathemerium*, becomes established in the canary in the absence of the sporozoite phase, highly parasitized blood was injected intravenously into clean (malaria-free) canaries. When numbers of parasites from 100,000 to one million were given essentially simultaneously with a maximally tolerated intravenous dose of pentaquine,* namely, 150 μg per bird (table 1), infection became established in all of the birds in spite of the well known effectively suppressive antimalarial activity of the drug. Intensive therapy to the limits of tolerance of the birds in this category does not effect a cure or prevent the infection from becoming established in the host.

In view of the fact that blood of canaries with a latent malarial infection is consistently infective on intravenous injection into clean birds, such blood must of course contain parasites. These relatively few parasites can be rendered noninfective to a recipient bird if the recipient bird is given an intravenous injection of the drug promptly after the intravenous inoculation.† However, the administration of the same drug to the "latent" bird, serving as a donor, as stated above, does not cure its latent infection. Consequently, the condition or location of some of the parasites in the "latent" bird must be different from that of clean birds having just received blood of a bird with an established infection.

If a small number of nonpoisoned parasites, e.g., 5000 or less, are injected intravenously into a clean bird and followed within five to ten minutes by a full dose of pentaquine, also administered intravenously, usually no infection occurs (table 1). It is our experience that this same number of normal parasites injected intravenously and not closely followed or preceded by drug is invariably infective to clean birds. As a matter of fact, we have confirmed the observation that ten, five, or even one normal trophozoite, are adequate to establish an infection when administered intravenously.[3] Since canaries, receiving small numbers of parasites and an effective dose of the drug soon after inoculation, remain uninfected, it is obvious that they can effectively dispose of this small number of parasites after treatment, in contrast to the condition after the reception of large numbers of parasites, in which circumstance some capacity of the host must have been exceeded, leading to an active infection resulting from some of the still viable parasites.

In order to check this point directly and experimentally in another manner, large numbers of parasites (around a million) were injected intravenously into a clean bird, serving as a "living test tube." A few minutes thereafter a

* Supplied through the courtesy of The Eli Lilly Co.

† This experiment on three recipient birds was kindly performed for us by R. W. Loehning, of this department. None of the recipients became infective.

3. Coulston, F. and Manwell, R. D. 1941, Single-parasite infections and exoerythrocytic schizogony in *Plasmodium circumflexum*. Am. J. Hyg. Sec. C. **34**: 119–125.

full dose of the drug also was given intravenously. Later, blood was taken from this recipient bird and a total cell count and percentage infection determined, so that by dilution of the blood any desired number of these in vivo-poisoned parasites could be administered to clean birds. These parasites had been exposed, within the hitherto clean bird serving as a "living test tube," to an intravenously injected dosage of 150 μg of pentaquine. After one hour blood was withdrawn and the count of parasitized cells was made. Appropriate dilutions were then made and known numbers of parasites were injected into other clean birds. Infection occurred consistently (table 2). This indicates that the parasites in the circulating blood of a previously clean bird, exposed to drug for a period of one hour, were not sufficiently injured as to prevent infectivity.

On the other hand, if parasites, exposed for a period of four hours in the donor bird serving as a "living test tube," were removed and injected intravenously to clean birds in small numbers, such as a total of 3000, no infection occurred in 30 out of 37 recipient birds in the series (table 3). Larger numbers of parasites of the same lot produced an incidence of infection roughly proportional to these numbers; and finally, when approximately 250,000 parasites were injected, practically all of the recipient birds developed an infection. This evidence supports the concept that large numbers do infect, whereas small numbers of the same lot of the in vivo-poisoned parasites do not. The data obtained (table 3), on being subjected to analysis,* prove to be beyond the likely probability of the chance occurrence of one or a few viable and resistant parasites having been unaffected by the drug, hence presumed to be responsible for the infection when large numbers of these parasites were injected.

TABLE 2.—*Results of intravenous inoculation into unparasitized birds of fixed numbers of parasites which had been subjected to the action of the drug for one hour in vivo by an intravenous injection of 150 μg of pentaquine.*

Number of birds	Number of parasites injected intravenously	Infected birds	Noninfected birds
3	1,800	3	0
3	18,000	3	0
1	36,000	1	0
2	183,000	2	0

TABLE 3.—*Results of the intravenous inoculation into unparasitized birds of fixed numbers of parasites poisoned in vivo in a donor bird for four hours by an intravenous injection of 150 μg of pentaquine.*

Number of birds	Approximate number of in vivo-poisoned parasites inoculated	Infected birds	Non-infected birds
37	3,000 or fewer	7	30
6	5,000 to 10,000	3	3
11	15,000 to 50,000	8	3
23	70,000 to 90,000	17	6
7	100,000 to 200,000	2	5
10	250,000 or more	10	0

The situation described above definitely leads to the concept of significance of numbers. Large numbers of parasites, e.g., 500,000 or more, when given intravenously with or followed very shortly by an intravenous injection of the drug in a maximally tolerated dose, always result in an infection which is usually evident only after a delay of 10 to 15 days. Thus, the intravenous injection of large numbers of parasites, followed by drug, appears to exceed or exhaust some element of the antimalarial capacity of the host, as a result of which an infection is established.

It should be stated at this point that in this type of experiment one often obtains markedly depressed curves of

* The Chi square function of the fourfold table gave the probability of 0.00298, which means one chance of infection in 333 experimental animals. The writers wish to thank Dr. J. A. E. Eyster for kindly making these calculations for us.

TABLE 4.—*Results of intravenous inoculation of parasites into unparasitized birds followed by an intravenous injection of 150 μg of pentaquine from 2 to 48 hours after the intravenous administration of parasites.*

Time in hours between inoculation and injection of drug into donor birds	Number of birds	Number of parasites injected	Infected birds	Non-infected birds
0	27	5 to 10,000*	0	27
2	22	29 to 10,000	10	12
4	17	237 to 5,000	17	0
5	6	176 to 4,080	6	0
11	2	29 to 290	2	0
24	17	3 to 2,600	16	1
48	3	17 to 1,700	3	0

* From table 1.

parasitemia, indicating the development of a partial immunity.

Parasites poisoned in vivo can be destroyed on injection into clean birds by the combination of actions of the antimalarial drug and of the defensive forces of the host, particularly if the numbers of parasites are small (table 1). "Latent" birds, which carry infective parasites in their active circulation, have not as yet been cured by any readily tolerated dosage of any antimalarial drug. They therefore must harbor in their bodies parasites which are either inaccessible or have become resistant to the drug and to the defensive forces of the host. Yet blood of "latent" birds subjected to in vivo action of the drug in a noninfected bird can be rendered noninfective, as stated above. The exposure to the drug, to be effective, must continue over one hour to render the parasites noninfective. It is then obvious that in birds with latent infections, parasites must be entering the blood stream more or less constantly, and perhaps in dribbling fashion. This means that those parasites which enter the blood stream (from hidden foci) shortly before the blood is taken may have had only a short period of exposure to the circulating blood and its content of antimalarial drug, and perhaps even to opsonins or antibody-like substances, and consequently, on prompt injection into a parasite-free and drug-free recipient, readily establish an infection.

If normal trophozoites, even in small numbers, are given intravenously three to four hours prior to the intravenous injection of the drug, an infection develops, indicating that within this period of time in vivo, at least some of the parasites have become inaccessible to otherwise effective or sterilizing concentrations of the drug (tables 4 and 5). Have these parasites become exoerythrocytic in this short time, or have some of them become hidden in locations within tissues in such a way as to be safe from the toxic action of the drug and from fixed phagocytes, or could they have acquired within this time a high grade refractoriness towards the drug?

We have observed that when a few hundred to a few thousand parasites

TABLE 5.—*Results of intravenous inoculation into unparasitized birds (group B) with blood from birds (group A) which had previously received an intravenous injection of a fixed number of normal parasites and at a subsequent stated period had received intravenously a dosage of 150 μg of pentaquine. In vivo poisoning of parasites occurred in group A.*

Exp. no.	Group A Number of parasites	Time in hours between inoculation and administration of drug	Time in hours between administration of drug and bleeding	Results in group B*
1	500	No drug		+
2	500	No drug		+
3	650	2	2	−
4	650	4	2	−
5	550	2	7	−
6	550	4	5	−
7	1,800	4	5	−
8	1,800	4	24	−
9	1,800	4	48	−
10	3,360	4	20	−
11	5,000	4	20	−
12	5,000	4	43	−
13	6,700	4	43	−

This table illustrates the noninfectivity of blood of group A birds for group B birds, while the group A birds themselves revealed a state of infection 6 to 12 days later.

* +Infected, − not infected.

were injected intravenously into clean birds and then the drug given by the same route four or more hours later, blood withdrawn between the 4- and 48-hour period was noninfective to other birds, yet these treated donor birds themselves developed an infection which became evident some days later (table 5). This observation indicates that those parasites which may have remained in the circulating blood are injured or are so affected by the drug that they are destroyed in some manner or other, if not in too large numbers, whereas an unknown number leave the active blood stream within the period of approximately four hours to become protected in some unknown manner against the actions of the drug. They survive, and in the course of two or three days some viable parasites gain access to the blood stream, again rendering it infective, as proven by the development of an evident infection when administered intravenously to clean birds. These hidden parasites evidently multiply so that some of the accumulated mass of new parasites succeed after two or three days in re-entering the active circulation so as to render it infective.

DISCUSSION

The course of events presented above furnishes the basis for at least a partial explanation of the latency of *P. cathemerium* infection in canaries. An easy assumption, which speaks in its favor, is that the parasites become hidden in tissues, perhaps in capillaries temporarily thrombosed or blocked by sludge, so that they become protected against direct and continued exposure to the antimalarial agent employed. To become fatally injured in these hypothetically walled-off capillaries, the parasites might require the penetrating power of drug concentrations beyond those tolerated by the host.

SUMMARY AND CONCLUSIONS

Infective malarial parasites (*Plasmodium cathemerium*) injected intravenously into clean birds establish a practically incurable infection within a period of four hours.

Small numbers of parasites can be poisoned by treatment in vivo, so that when administered to clean birds they do not infect, whereas large numbers of the same lot do. This fact indicates that large numbers exhaust some necessary antimalarial humoral factor leading to susceptibility to active infection.

Blood of birds having a latent infection, injected intravenously into clean birds, can be rendered noninfective in these recipients by prompt intravenous injections of the drug employed in this work.

Since the blood stream of birds given small numbers of parasites is rendered noninfective by immediate intravenous treatment with the drug, and since the blood stream of birds with a latent infection having approximately the same number of parasites and given intensive intravenous treatment continues to be infective, it would appear that there are parasites in the latent bird that are inaccessible to the drug and that the progeny of these hidden parasites are continually entering the active blood stream.

STUDIES OF THE EFFECT OF ANTIBIOTICS ON INFECTIONS WITH THE MOUSE PINWORM, *ASPICULURIS TETRAPTERA*

III. THE ACTIONS OF AUREOMYCIN, BACITRACIN AND POLYMYXIN B

HELEN S. WELLS

From the School of Public Health, Columbia University, New York City

In previous reports (Wells, 1951a and b) the actions of terramycin hydrochloride, neomycin, chloramphenicol and dihydrostreptomycin on experimental infections with the mouse pinworm, *Aspiculuris tetraptera*, have been presented. It was found that terramycin reduced the worm burden and stunted remaining worms. Neomycin chloramphenicol and dihydrostreptomycin when given orally, on the other hand, resulted in an increased worm yield of normal sized worms. In the present report the findings with aureomycin, bacitracin and polymyxin B are presented.

METHODS AND MATERIALS

The techniques used in this study were the same as those employed in the previous studies. Helminth-free mice of approximately 30 days of age were infected orally with 300 embryonated eggs of *A. tetraptera*. In the initial experiments daily oral doses of 160 mg/kg of aureomycin were administered. In subsequent experiments the dosage employed was 500 mg/kg. The daily oral dosage of bacitracin was 20,000 units/kg and for polymyxin B, was 500 mg/kg. The mice were autopsied at various intervals after infection and treatment. The entire large intestine was examined microscopically and the worms recovered were counted. The measurement of the lengths of a sample of worms was also made.

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The author wishes to thank Dr. H. W. Brown for advice in the execution of this problem and the preparation of the manuscript.

Aureomycin

Experiment 1.—Treated mice received 160 mg/kg of aureomycin. In experiment 1a treatment was initiated 2 days prior to infection and continued for 16 days. The day after receiving the last dose of aureomycin the mice were autopsied and the worms were counted (i.e., 14 days after infection). Treated animals harbored an average of 105 worms and the controls 130, a difference which is not statistically significant. The average length of worms in the treated animals was 1.24 mm and in the control animals 1.55 mm, a significant difference. In experiment 1b treated animals received similar dosage of aureomycin beginning 7 days after infection and continued for 7 days. The average worm yield was 129 in the treated animals as compared with 130 in the control animals. The average length of worms in treated animals was 1.34 mm, which is significantly smaller than the average length of 1.55 mm of the controls.

Experiment 2.—The recovery of smaller worms from treated animals in experiment 1 indicated that aureomycin has some activity against *Aspiculuris*. Therefore, experiments were undertaken with larger doses of the antibiotic, and in all experiments other than experiment 1, 500 mg/kg of aureomycin were administered daily. In experiment

2a mice received 16 daily doses of aureomycin beginning 2 days prior to infection. On autopsy the average worm yield in the treated animals was only 7, while the controls harbored 57, a highly significant difference. The average length of worms in treated animals was 0.95, which is significantly shorter than the 1.46 mm average length of worms in controls. In experiment 2b treated mice received 7 daily doses of aureomycin, beginning 7 days after infection. The average worm yield in the treated mice was 50, which was not significantly different from the control animals, which averaged 57 worms. The average length of worms in treated animals was 1.10 mm, which is significantly shorter than the 1.46 mm average length of worms from the controls.

Experiment 3.—This experiment is a duplicate of experiment 2. In experiment 3a the treated animals, after receiving 16 daily doses of aureomycin, harbored an average of 67 worms, which was significantly lower than the control yield of 102. The average length of worms in treated animals was 0.83 mm, and that of the controls 1.42 mm, a significant difference. There were 3 deaths among 15 treated animals in the 2nd week of treatment, indicating that the dosage level employed was toxic. In experiment 3b treated mice, after receiving 7 doses of aureomycin, harbored an average of 41 worms, which was also significantly lower than the number found in the controls. The average length of worms from treated animals was 1.17 mm, which was significantly smaller than the worms from control animals.

Experiment 4.—This experiment is also a duplicate of experiment 2. In experiment 4a treated mice, after receiving 16 daily doses of aureomycin, harbored an average of 5 worms as compared to 118 worms in control animals, a highly significant difference. The average length of worms in treated animals was 0.75 mm and in control mice 1.29 mm, a highly significant difference. In experiment 4b treated mice, after receiving 7 daily doses of aureomycin, harbored an average of 5 worms, identical with the yield from animals receiving the 16 day treatment. The average length of worms in the

TABLE 1.—*Effect of aureomycin on* A. tetraptera *worm burden in mice.*

Exp. no.	Treated mice					Control mice		t*
	Number of mice	Daily dosage, mg/kg	Day of infection treatment was initiated	Total days of treatment	Average worm burden	Number of mice	Average worm burden	
1a	15	160	−2	16	105	15	130	1.7
1b	15	160	7	7	129			0.08
2a	15	500	−2	16	7	15	57	6.1†
2b	15	500	7	7	50			0.7
3a	12	500	−2	16	67	14	102	2.6†
3b	15	500	7	7	41			3.9†
4a	15	500	−2	16	5	15	118	9.5†
4b	15	500	7	7	5			9.4†
5	15	500	7	14	5	15	100	9.1†
6	12	500	14	14	72	14	154	5.4†

All mice received 300 eggs.
* Difference in means/standard error of the difference in means.
† Indicates significant difference between treated and control animals, with $P<0.01$.

treated animals was 0.83 mm, significantly smaller than worms from control animals.

Experiment 5.—Experiments 5 and 6 were designed to determine the effect of aureomycin on older worms. In experiment 5 aureomycin was administered 7 days after infection and continued for 14 days. Mice were autopsied 21 days after infection. The average worm yield in treated animals was 5 as compared with a control yield of 100. Worm measurements were made only of females because by this time in the life cycle the male and female worms differ in size. In treated animals the average female worm length was 1.26 mm and those from control mice 3.31 mm, a significant difference.

Experiment 6.—In experiment 6 treatment was begun 14 days after infection and continued for 14 days after. Mice were autopsied after 28 days of infection at a point in the life cycle when normally female worms are gravid and eggs are passed in the feces. The average worm yield in treated animals was 72, and in controls 154, a significant difference. In treated animals the average worm length was 3.40 mm and that of the control mice was 4.15 mm, a significant difference. Three of 15 treated mice died during the 2nd week of therapy, confirming that the dose employed was toxic. Of the 12 treated animals, 1 contained no female worms, 3 contained females with no eggs, and 7 contained gravid females with uteri partially filled with eggs. All worms from control animals were fully gravid. Eggs from worms of treated mice became embryonated on incubation.

TABLE 2.—*Effect of aureomycin on lengths of* A. tetraptera.

Exp. no.	Treated mice		Control mice		t*
	Number of worms measured	Average worm length (mm)	Number of worms measured	Average worm length (mm)	
1a	50	1.24	50	1.55	8.6†
1b	50	1.34			4.2†
2a	33	0.95	50	1.46	9.4†
2b	50	1.10			3.8†
3a	50	0.83	50	1.42	12.4†
3b	40	1.17			4.6†
4a	25	0.75	50	1.29	18.0†
4b	22	0.83			6.8†
5	29	1.26	48	3.31	11.7†
6	41	3.40	50	4.15	5.0†

In each experiment worms from 5 animals were measured. Wherever possible 10 worms were measured from each animal
See footnotes under table 1.

Bacitracin

Experiment 7.—The treated mice received bacitracin in 16 daily, oral doses of 20,000 units/kg, beginning 2 days prior to infection with *Aspiculuris*. On autopsy 14 days after infection the average worm yield in treated animals was 30 and in the controls 80, a significant difference. The average length of worms from treated animals was 0.79 mm and in controls 1.44 mm, a significant difference.

Experiment 8.—Treatment was initiated 7 days after infection and continued for 7 days. The average worm yield in treated animals was 95, which was higher than that of the control yield of 80 but was not significantly different. The average length of worms

TABLE 3.—*Effect of bacitracin on* A. tetraptera *worm burden in mice.*

Exp. no.	Treated mice				Control mice		t*
	Number of mice	Day of infection treatment was initiated	Total days of treatment, 20,000 units/kg daily	Average worm yield	Number of mice	Average worm yield	
7	15	−2	16	30	15	80	4.5†
8	15	7	7	95	15	80	1.0
9	15	7	14	8	15	31	3.1†
10	15	14	14	21	15	34	1.7

All animals received 300 eggs.
See footnotes under table 1.

from the treated animals was 1.09 mm, significantly smaller than worms from controls, which average 1.44 mm.

Experiment 9.—Treatment was begun 7 days after infection and continued for 14 days. At autopsy 21 days after infection, the average worm burden in treated mice was 8, significantly less than the controls' yield of 31. The average length of female worms from treated animals was 1.75 mm, significantly smaller than the average length of 3.15 mm of worms from control mice.

Experiment 10.—Treatment was initiated 14 days after infection and continued for 14 days. On autopsy 28 days after infection the average worm yield in treated animals was 21, not significantly different from the controls' yield of 34. The average length of female worms from treated animals was 3.67 mm and that of the controls 4.18 mm, a significant difference. All of the control mice contained gravid female worms. In contrast of 15 treated animals, 3 harbored no females, 1 contained females with empty uteri, 2 contained females with partially filled uteri and 9 harbored females with uteri packed with eggs. Eggs from treated animals became embryonated on incubation.

Polymyxin B

Experiment 11.—Treated mice received 16 daily doses of 500 mg/kg of polymyxin B, beginning 2 days prior to infection. On autopsy 14 days after infection, the average worm yield in treated animals was 28, not significantly different from that of the control mice yield of 39. The average length of worms from treated animals was 1.27 mm, which was significantly smaller than the average of 1.38 mm of worms from the control animals.

Experiment 12.—Treatment was initiated 7 days after infection and continued for 7 days. The average worm yield in treated animals was 32, not significantly different from the control yield of 39. The average length of worms from treated animals was 1.31, not significantly different from the average length of 1.38 mm of worms from control animals.

TABLE 4.—*Effect of bacitracin on lengths of* A. tetraptera.

Exp. no.	Treated mice		Control mice		t*
	Number of worms measured	Average worm length (mm)	Number of worms measured	Average worm length (mm)	
7	46	0.79	50	1.44	13.0†
8	50	1.09	50	1.44	11.7†
9	33	1.75	41	3.15	11.6†
10	44	3.67	50	4.18	5.6†

In each experiment worms from 5 animals were measured. Wherever possible 10 worms were measured from all animals.
See footnotes under table 1.

TABLE 5.—*Effect of polymyxin B on* A. tetraptera *worm burden in mice.*

Exp. no.	Treated animals				Control animals		t*
	Number of mice	Day of infection treatment was initiated	Total days of treatment 500 mg/kg daily	Average worm yield	Number of mice	Average worm yield	
11	15	−2	16	28	15	39	1.5
12	15	7	7	32	15	39	0.9

All mice received 300 eggs.
See footnotes under table 1.

DISCUSSION

Aureomycin, bacitracin and polymyxin B show action similar to that exhibited by terramycin (Wells, 1951a) in *A. tetraptera* infections in mice. With oral therapy aureomycin and bacitracin both reduce the worm burden, and the remaining worms are considerably reduced in size. Treatment with polymyxin did not reduce the worm burden but did result in a diminution of the size of worms in treated animals.

In doses of 500 mg/kg aureomycin appears to be equal to terramycin in activity. It is difficult to make detailed comparison of efficiency because in the case of aureomycin we were working in a toxic range. It is obvious, however, that aureomycin exerts a more rapid effect on the worm. Even with inadequate dosage at the rate of 160 mg/kg, aureomycin significantly reduced the worm's size in 7 days. In 2 of 3 experiments at a dosage level of 500 mg/kg, 7 days of treatment seemed to be as effective as prolonged treatment. In experiment 4 the worm burden in mice given 16 days of treatment was the same as those given 7 days of treatment, and there was little difference in size. Although only one experiment was conducted with terramycin, administered for 7 days at the rate of 500 mg/kg, the worms were found to be neither reduced in size nor number. Wells et al (1951) and Loughlin et al (1951) reported successful therapeutic trials with terramycin in the treatment of infection with the human pinworm *Enterobius vermicularis*. Unpublished clinical data (Shookoff and coworkers) indicate that aureomycin is also effective in the treatment of enterobiasis.

TABLE 6.—*Effect of polymyxin B on lengths of* A. tetraptera.

Exp. no.	Treated animals		Control animals		t*
	Number of worms measured	Average worm length (mm)	Number of worms measured	Average worm length (mm)	
11	49	1.27	50	1.38	4.4†
12	50	1.31	50	1.38	1.5

In each experiment worms from 5 animals were measured. Wherever possible 10 worms were measured from all animals.
See footnotes under table 1.

The effect of bacitracin on young larval forms is similar to that of terramycin and aureomycin. Older worms, however, were not appreciably affected by this antibiotic. The adult female worms, although smaller in size than the controls, did not show impairment of fecundity in 9 of 15 animals. Since fully gravid females developed during the course of treatment, it appears that bacitracin exerts a more superficial effect on the worm than either aureomycin or terramycin.

The results with polymyxin B are suggestive of the action of aureomycin with inadequate dosage. It is possible that increased dosage of polymyxin B might result in a more pronounced activity.

It should be emphasized that the antibiotics exert their most profound

effects on the young larvae of *A. tetraptera*. We do not know whether this holds true in infections with *E. vermicularis*. Loughlin et al (1951) report the recovery of dead eggs of *E. vermicularis* on scotch tape swabs taken during the first few days of therapy with terramycin. It is apparent that we are screening drugs agains *A. tetraptera* for use in human enterobiasis with a more resistant parasite than the human form.

SUMMARY

1. Data on the effects of aureomycin, bacitracin and polymyxin B on infections with the mouse pinworm, *Aspiculuris tetraptera*, are presented.

2. Aureomycin and bacitracin both decrease the worm burden and stunt remaining worms. Polymyxin B reduces the size of worms only.

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THE EFFECT OF ANTIBIOTICS AND METABOLITES ON THE IMMUNITY OF MOSQUITOES TO MALARIAL INFECTION

LEVON A. TERZIAN, N. STAHLER, AND P. A. WARD
From the Naval Medical Research Institute, Bethesda 14, Maryland

It has already been established that sulfonamides may significantly increase the innate susceptibility of an animal to a particular parasite[1] but that this effect depends upon a critical tissue level, measured by the concentration of the drug made available in the standard maintenance diet. Thus, it was shown, on the basis of changes in oöcyst density, that sulfanilamide and sulfadiazine administered in 0.1% concentration in the diet increased the susceptibility of the mosquito *Aëdes aegypti* to the avian malarial parasite *Plasmodium gallinaceum*, but that no significant effect could be produced when the concentration of these compounds was increased to 0.2%. The present paper is a continuation and an extension of that study.

MATERIALS AND METHODS

In these experiments, laboratory strains of *A. aegypti* and *Anopheles quadrimaculatus* were infected with the 8A strain of *P. gallinaceum*. The compounds to be evaluated were added in various concentrations to a 4% sugar solution used as the standard maintenance diet for infected mosquitoes. There was no mortality in either the control groups (4% sugar alone) or the experimental groups (4% sugar plus drug) during the time required to complete an experiment, and the mosquitoes fed readily on all solutions. The mosquitoes were set up in plastic cylinders in groups averaging 45 females, and for each experiment mosquitoes of the same age derived from the same larval colony were used. The sugar and drug solutions were made available to the adult mosquitoes 24 hours after emergence. Seventy-two hours after drug administration had been started the mosquitoes were exposed to a chick infected with *P. gallinaceum*. Each group of mosquitoes maintained on a specific drug diet was paired with its control group, and both groups were fed simultaneously on a single bird. This same bird was used as the source of infection for all the paired groups within an experiment. Following the blood meal, the unfed females were removed from the cylinders. Meanwhile, drug administration was usually continued until the completion of an experiment.

In evaluating the intensity of the parasitism in the mosquito the procedure was to count the number of oöcysts present on the entire stomach at a magnification of approximately 400 diameters. Since the amount of blood taken up by the mosquito determines the parasite dosage, and this could affect the level of parasitism, such variation was controlled by producing adult mosquitoes of nearly uniform size. Oöcyst counts were done on the fifth, sixth, and seventh day of the infection, the day of the infective blood meal being designated as 0 day.

RESULTS

The essential effect of the sulfonamides on the innate susceptibility of *A. aegypti* to infection by *P. gallinaceum* has already been demonstrated,[1] but since in these original experiments only two drug-diet concentrations were studied, it was considered necessary to determine the effects of these compounds through a wider range of concentrations. In figure 1, as in all similar figures, each point represents an individual mosquito, while the level of infection, or the total number of oöcysts present on the stomach wall of each, is shown by the vertical scale at the left of the figure. The

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The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1. Terzian, L. A. 1950, The sulfonamides as factors in increasing susceptibility to parasitic invasion. J. Infect. Dis. **87**: 285–290.

total number of points within a column represents the number of mosquitoes dissected from an experimental or a control group. To avoid repetitious reference to statistical procedures, it may be noted here that all differences between groups, cited in the text, were tested for significance by the method of chi square as well as by the Mathieson solution.[2] To facilitate discussion or illustration, differences have been occasionally cited in terms of median values alone, but these differences are actually based on the analyses indicated above.

In the typical experiment cited in figure 1, as in all the experiments with *A. aegypti*, each experimental group on a specific drug concentration was paired with a control group maintained on sugar alone. Each pair was fed simultaneously on a single infected chick, and the other paired groups within an experiment were fed in sequence on the same chick. The data of this experiment show that at a 0.01% concentration of sodium sulfadiazine there is no evident drug effect and thus no discernible increase in parasitism among the individuals in the experimental group 170-02 as compared to its control group 170-01. Consequently, the distribution according to oöcyst densities is approximately the same in both groups. With the concentration of sulfadiazine increased to 0.05% and 0.1%, however, the drug produced a pronounced increase in host susceptibility, a change which is evidenced by the significantly higher oöcyst counts of the individuals in the experimental groups 170-04 and 170-06 as compared to the oöcyst counts of the mosquitoes in the respective paired control groups 170-03 and 170-05. Thus, in both experimental groups the median oöcyst count was approximately 50

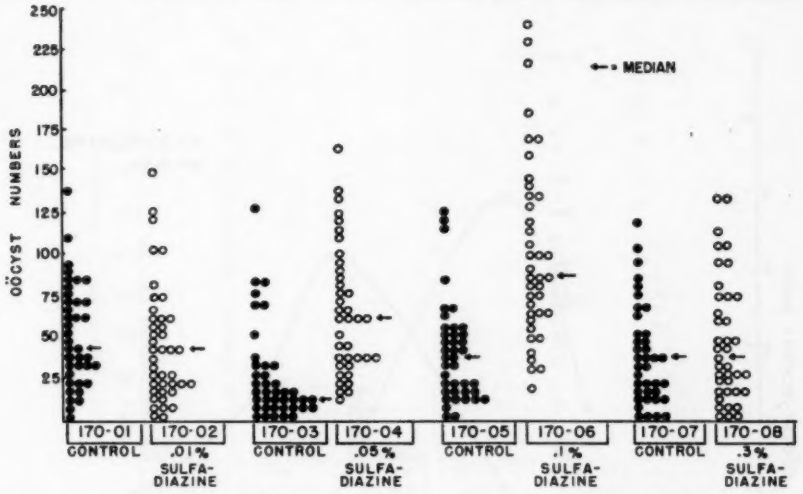

FIG. 1.—A comparison of the oöcyst densities of control groups of *A. aegypti* maintained on diets of 4% sugar and infected with *P. gallinaceum* and of simultaneously infected experimental groups maintained on 4% sugar with various concentrations of sodium sulfadiazine added.

2. Statistical Memo. No. 4, Methods of making experimental inferences, Frankford Arsenal. Sept. 1946.

oöcysts higher than the median counts in the control groups, indicating a marked increase in host susceptibility. It would appear that this effect of the drug on the host parasite balance is more pronounced at a 0.1% concentration than at a 0.05% concentration, but it should be noted that there was a considerable fluctuation in the control groups. The median count in control group 170-03 was 14 oöcysts, and in control group 170-05 it was 35.8 oöcysts. Therefore, although the difference between control and experimental groups was approximately 50 oöcysts in both cases, this difference represents more than a fourfold increase in experimental group 170-04 as compared to group 170-03, whereas this difference is a little more than a twofold increase in group 170-06 above that of the paired control group 170-05. Thus, although the drug produces a significant increase in host susceptibility at both concentrations, the maximum effect appears to be at a concentration of 0.05%. At a concentration of 0.3% sulfadiazine, however, which is the maximum tolerated dose on this regimen, the effect of the drug is essentially eliminated. There is no significant difference in distribution, on the basis of oöcyst densities, between the mosquitoes in experimental group 170-08 and the individuals in control group 170-07, and it is obvious that at this higher concentration the drug no longer has any significant effect on the susceptibility of the host. Thus, it is evident that (a) sulfadiazine has no effect on host susceptibility at drug con-

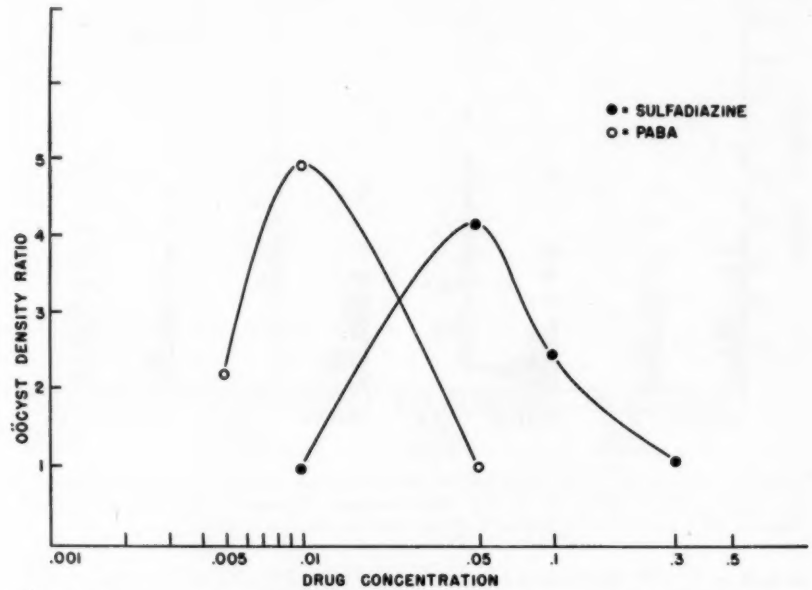

FIG. 2.—The concentration-action relations of sulfadiazine and para-aminobenzoic acid as shown by the fold increase in the median number of oöcysts in treated mosquitoes as compared to the median number of oöcysts in control mosquitoes with drug concentration on a logarithmic scale.

centrations below certain critical levels, (b) the maximal effect of the drug in this host-parasite relation depends upon a specifically limited range of drug concentrations, and (c) at concentrations sufficiently above these critical levels the effect of the drug is no longer evident and no significant change in host susceptibility can be produced.

sity level of all the mosquitoes within a group. Thus, these curves, based on comparative changes in oöcyst density between experimental and control groups, show that the maximal effect of these compounds depends upon a critical drug-diet concentration and that at higher concentrations this specific effect is significantly decreased or elimi-

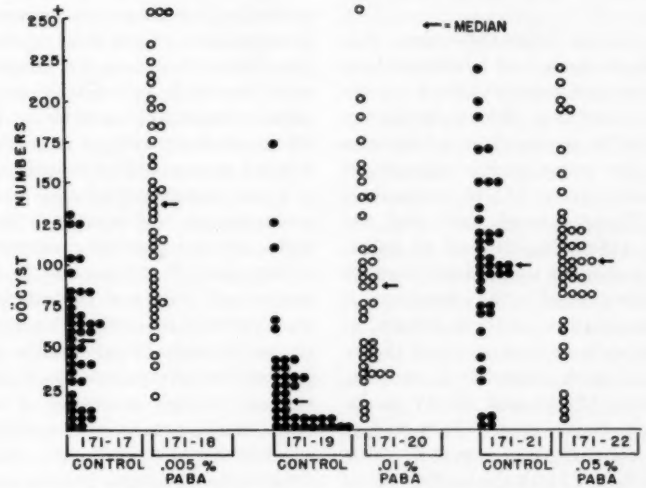

FIG. 3.—A comparison of the oöcyst densities of control groups of *A. aegypti* infected with *P. gallinaceum* and similarly infected experimental groups maintained on para-aminobenzoic acid.

This concentration-action relation, which has proven to be characteristic of all the compounds investigated thus far which elicit this specific effect on the the host-parasite association of *A. aegypti* and *P. gallinaceum*, is better illustrated by the concentration-action curves for sulfadiazine and for para-aminobenzoic acid shown in figure 2. In this figure the density ratio is the ratio of the median number of oöcysts found in the experimental group to the median number of oöcysts found in the control group, the median having proven to be, for certain purposes, the best single representation of the oöcyst density.

nated. It will, of course, require additional points to define exactly these concentration-action curves, but the data from numerous experiments make it abundantly clear that these curves in figure 2 represent the essential characteristics of the concentration-action relations of these compounds in producing their effects on host susceptibility or resistance.

It has already been shown that para-aminobenzoic acid, administered in excess along with effective concentrations of sulfadiazine, antagonizes the action of sulfadiazine and nullifiies its effects on the susceptibility of the host.[1]

In view of this fact, and in view of the accepted theoretical relation between the sulfonamides and para-aminobenzoic acid,[3] it is important to note that in this host-parasite relation, para-aminobenzoic acid in proper concentration produces an effect on the host-parasite equilibrium similar to the effects produced by the sulfonamides. This is shown by the experiment presented in figure 3.

It is evident from these data that para-aminobenzoic acid administered in certain critical concentrations in the mosquito diet has produced a significant increase in the susceptibility of the mosquito to the parasite. For example, in experimental group 171-18, maintained on 0.005% para-aminobenzoic acid, and in group 171-20, maintained on 0.01% para-aminobenzoic acid, there is an extreme shift upward in the oöcyst counts of the mosquitoes in these groups, as compared to the oöcyst counts of the individuals in their respective paired control groups, 171-17 and 171-19, maintained on sugar alone. The median count in control group 171-17 is 59 oöcysts, while in 171-18 the median count is 134.5 oöcysts; and in control group 171-19 the median is only 17 oöcysts, whereas in 171-20 the median has risen to 86.2 oöcysts. Thus, in both experimental groups the parasitism is significantly higher and far more intense than in the respective control groups. On the other hand, at a concentration above these critical levels the effect of the drug has been either depressed or eliminated and the distribution according to oöcyst density is the same in the experimental group as it is in the control group. Consequently, in group 171-22, maintained on a concentration of 0.05% para-aminobenzoic acid, the median count is 103.5 oöcysts, while in the control group 171-21 the median count is 102.8 oöcysts, and it is obvious that at this level the drug no longer exerts any discernible effect on the host-parasite balance. Thus, para-aminobenzoic acid, like the sulfonamides, is able in certain critical concentrations to produce changes in host-parasite equilibrium resulting in a pronounced increase in the susceptibility of the host to the parasite. Here, therefore, is a unique situation in which sulfadiazine alone and para-aminobenzoic acid alone produce identical effects and yet in combination behave as competitive inhibitors.

Even though sulfadiazine and para-aminobenzoic acid in critical concentrations both increase the susceptibility of *A. aegypti* to *P. gallinaceum*, there is experimental evidence to indicate that they produce this effect through different mechanisms. To determine whether or not these compounds might be functioning through a related, if not the same, mechanism to produce this specific effect on host immunity, they were administered together in concentrations below the minimal effective levels of each but which together might be assumed to approximate a critical or effective concentration. Thus, 0.01, 0.02, 0.025 and 0.03% concentrations of sulfadiazine were administered, in the order given, along with 0.001, 0.002, 0.0025 and 0.003% concentrations of para-aminobenzoic acid, according to the usual regimen. There was no discernible evidence in any of these experiments of an additive effect of these two compounds since there were no differences in distribution according to oöcyst density between control and experimental groups. If it is assumed that compounds related in their modes of action either potentiate one another or produce an additive effect, then, on the basis of

3. Woolley, D. W. 1947, Recent advances in the study of biological competition between structurally related compounds. Physiol. Rev. **27**: 308–333.

these experiments it appears that in producing this specific effect on host susceptibility, sulfadiazine and para-aminobenzoic acid function through independent mechanisms.

It may be of interest to digress momentarily to illustrate the extreme variation in infectivity to mosquitoes which may occur during short intervals of time in chicks parasitized by *P. gallinaceum*. It is this variability that makes it necessary always to pair a control group of mosquitoes with an experimental group and to infect both groups simultaneously. In experiment 171, as in the other experiments, a pair of control and experimental groups was fed simultaneously and then each additional pair in the experiment was fed in sequence on the same chick. For example, the mosquitoes of groups 171-17 and 171-18 were fed first, then 170-19 and 171-20 and finally 171-21 and 171-22, all on chick number 1996. Ordinarily, all the mosquitoes within a cylinder that are going to take a blood meal will have engorged at the end of ten minutes. Transferring the chick from one pair of cylinders to the next may require an additional five minutes. It will usually require, therefore, one hour at most to infect the four pairs of cylinders of a typical experiment. In this particular experiment, the total time elapsed between the start and finish of the feedings was approximately 40 minutes. During that short interval, there was a striking and continued fluctuation in the infectivity of the chick to the mosquitoes, evidenced by the extreme changes in distribution according to oöcyst density in each of the control groups. Thus, in a matter of 40 minutes, the median oöcyst count dropped from 59 oöcysts in group 171-17 to 17 oöcysts in group 171-19, and then soared to 102.8 oöcysts in group 171-21. Such fluctuations, although usually not as extreme as in this case, have occurred in many experiments. It is obvious, therefore, that these changes in the infectivity of chicks infected with *P. gallinaceum*, to the mosquito *A. aegypti*, cannot be due, as it has been suggested,[4] to changes in the gametocytes related to the stage of the infection. Nor can these fluctuations in infectivity be due to the depletion of blood substances of the chick,[5] since the mosquitoes of group 171-21, fed last in this series, developed the highest oöcyst counts of the control series, even though by this time the chick had been considerably weakened. Nor is this fluctuation in infectivity related to the body temperature of the chick, for its temperature may drop steadily as much as 10 degrees F during the period it is being dexposed to the mosquitoes; and yet, during that interval of decreasing body temperature, its infectivity to the mosquitoes may increase, decrease, or remain comparatively constant, following no consistent pattern related to the changes in body temperature. Thus far, the factors which determine these marked variations in infectivity of the chick to the mosquito remain obscure. It appears, however, that the oöcyst density is simply a function of the number of gametocytes taken up in the blood meal and that the variations in oöcyst numbers from group to group may be due rather to rapidly occurring blood volume and circulatory changes in the chick. The range in oöcyst counts within a given group of mosquitoes is determined, of course, by individual dif-

4. Lumsden, W. H. R. and Bertram, D. W. 1940, Observations on the biology of *Plasmodium gallinaceum* in the domestic fowl, with special reference to the production of gametocytes and their development in *Aëdes aegypti*. Ann. Trop. Med. **34:** 135–160.
5. Cantrell, W. and Jordan, H. B. 1946, Changes in the infectiousness of gametocytes during the course of *Plasmodium gallinaceum* infections. J. Infect. Dis. **78:** 153–159.

ferences in innate susceptibility or resistance. Weight measurements of the mosquitoes before and after feeding have shown no significant differences in the amount of blood taken up by the individuals within a group, and careful gametocyte counts of the mosquito stomach contents, made immediately after feeding, have shown a uniform distribution of parasites throughout the nobenzoic acid is independent of the variability illustrated above. In another experiment, the distribution of mosquitoes according to oöcyst density showed little variation and was essentially the same in all the control groups. Nevertheless, the mosquitoes maintained on 0.005 and 0.01% concentrations of para-aminobenzoic acid showed more than twofold and threefold increases in oö-

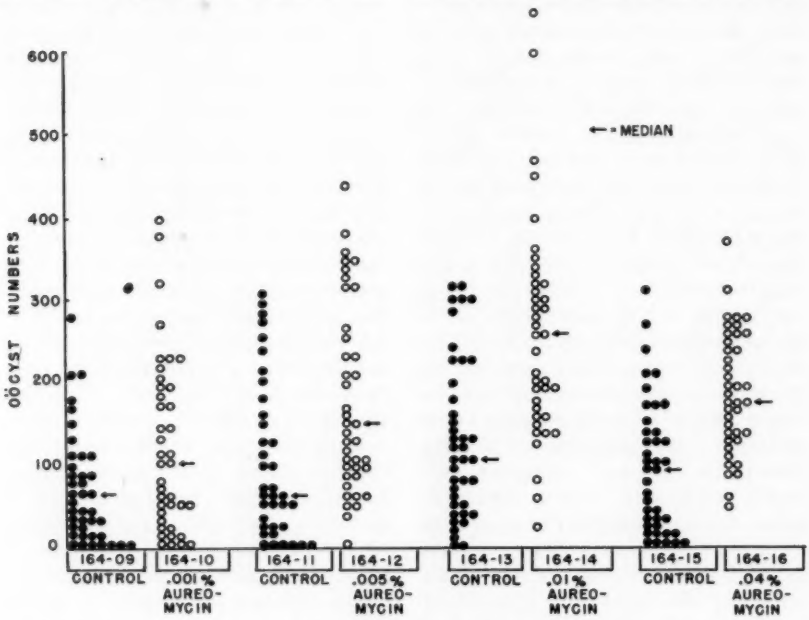

FIG. 4.—A comparison of the oöcyst densities of control groups of *A. aegypti* infected with *P. gallinaceum* and similarly infected experimental groups maintained on aureomycin.

group. It may be assumed, therefore, that the inoculum or the parasite dosage taken up in the blood meal is reasonably uniform for all the mosquitoes within a group and that the differences in the resulting oöcyst counts are due essentially to differences in individual susceptibility or resistance.

It is important to note that the increase in susceptibility of *A. aegypti* to *P. gallinaceum* produced by para-aminocyst counts above those of the individuals in the paired control groups. And again, at a concentration of 0.05%, the effect of the drug was eliminated and there was no discernible effect on the host-parasite equilibrium.

The antibiotic aureomycin, like the sulfonamides and para-aminobenzoic acid, produces a similar effect on the host-parasite equilibrium by increasing the host susceptibility to infection. This

is demonstrated by the experiment cited in figure 4. From these data it is clear that in a concentration of 0.01% in the mosquito diet, aureomycin has markedly increased the susceptibility of *A. aegypti* to *P. gallinaceum*. Whereas the median count in control group 164-13 is 119 oöcysts, the median count in experimental group 164-14 is 264.5 oöcysts. Furthermore, while in the control group the highest individual count was 325 oöcysts, in the experimental group there were 11 individuals with counts above this figure, and the highest single count was over 650 oöcysts. In general, except that aureomycin elicits its maximum effect at a different concentration level, the pattern of its activity is similar to that of the sulfonamides and para-aminobenzoic acid. At a concentration of 0.001% there is some effect on the host-parasite equilibrium, evidenced by the shift upward in the oöcyst densities of the mosquitoes in group 164-10 as compared to the control group 164-09. At a concentration of 0.005%, aureomycin begins to manifest its effects on host susceptibility more markedly, producing a still further shift upward in the oöcyst densities of the individuals in experimental group 164-12. Administered in a concentration of 0.01%, the drug produces its maximal effect, as previously indicated. With concentration increased to 0.04%, however, the effect of the drug has begun to decline even though the oöcyst numbers of the mosquitoes in group 164-16 are still significantly higher than the oöcyst numbers of those in the paired control group. Thus, like the sulfonamides and para-aminobenzoic acid, aureomycin is effective in increasing host susceptibility but only at certain critical concentrations, and in excess of these levels its effect is significantly depressed or eliminated.

On the other hand, in spite of the similarity of effect and concentration-action relation, the experimental evidence available at this stage of the study indicates that aureomycin exerts its effect through mechanisms apparently unrelated to the mode of action of either the sulfonamides or para-aminobenzoic acid. Thus, 0.01% aureomycin, when administered in combination with 0.2% para-aminobenzoic acid, shows no loss in activity and produces the same degree of increase in host susceptibility as when it is administered alone, unlike the apparent competitive inhibition which occurs when para-aminobenzoic acid in excess is administered in combination with critical concentrations of sulfadiazine.[1] Furthermore, there is no evidence of an additive effect when aureomycin in subminimal concentrations is administered along with subminimal concentrations of para-aminobenzoic acid. Thus, 0.001% aureomycin in combination with 0.001% para-aminobenzoic acid, a total concentration which might be assumed to have reached a critical level produces no demonstrable increase in the oöcyst densities of the experimental groups as compared to the control groups. It appears, therefore, that in spite of the fact that aureomycin, the sulfonamides, and para-aminobenzoic acid all produce in critical concentrations the same marked increase in host susceptibility by the same type of action pattern, nevertheless each compound apparently elicits its effect through an individual mode of action.

Streptomycin, also, administered in critical concentrations produces a marked increase in host susceptibility. At a concentration of 0.001% there is a significant increase in susceptibility, evidenced by the greater oöcyst numbers of the mosquitoes of the experimental groups as compared to the oöcyst levels of the mosquitoes in the control groups. Streptomycin does not produce as marked an effect as aureomycin, the sul-

fonamides, or para-aminobenzoic acid, but its effect is evident nevertheless, producing in some experiments more than a two-fold increase in the oöcyst numbers of the experimental groups as compared to the oöcyst numbers of the control groups. At a concentration of 0.005% and at higher concentrations, streptomycin no longer has any effect, and experimental groups maintained on

Thus, in the experiment cited in figure 5 the mosquitoes maintained on critical concentrations of folic acid developed significantly lower levels of parasitism when exposed to infection than did the normal control individuals maintained on sugar alone. For example, in control group 174-17 there were only three individuals with counts below 50 oöcysts, the least infected animal having a count

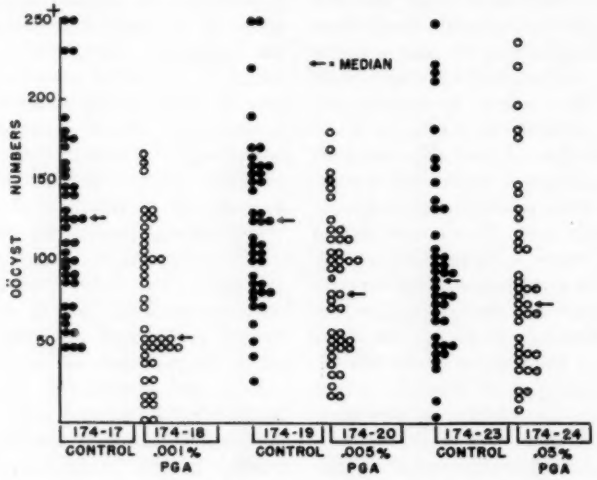

FIG. 5.—A comparison of the oöcyst densities of control groups of *A. aegypti* infected with *P. gallinaceum* and similarly infected experimental groups maintained on folic acid.

these higher concentrations, and control groups, have the same level of infection.

All the compounds evaluated thus far in this host-parasite relation have been characterized by an ability to increase host susceptibility so that mosquitoes maintained on these factors, when exposed to infection, have uniformly developed higher infections than the mosquitoes maintained on sugar solutions alone. It is noteworthy, therefore, that pteroylglutamic acid, or folic acid, has an exactly opposite effect and is characterized, instead, by an ability to increase host resistance.

of 45 oöcysts, whereas in experimental group 174-18, maintained on 0.001% folic acid, there were 16 mosquitoes with counts below 50 oöcysts, the counts ranging from 0 to 50 oöcysts. The striking shift downward in the oöcyst counts of the mosquitoes maintained on 0.001% folic acid is further evidenced by the fact that the median count in group 174-17 was 120.8 oöcysts, whereas the median count in experimental group 174-18 dropped to 54 oöcysts. It is obvious, therefore, that at this concentration the effect of folic acid on the host-parasite equilibrium has resulted in a significant increase in host resistance, characterized

by the significant reduction in oöcyst numbers of the drug-fed mosquitoes. At a concentration of 0.005%, folic acid still produces a significant increase in host resistance although its effect has obviously declined. Thus, the median count in control group 174-19 is 119 oöcysts, while in experimental group 174-20 the median count is 84 oöcysts, which represents a difference that is significant but not as marked as the differences between control groups and experimental groups maintained on 0.001% folic acid.

these experiments were repeated using the same parasite but with *Anopheles quadrimaculatus* as the host animal. To determine the basic differences in infectivity to *P. gallinaceum* between *A. quadrimaculatus* and *A. aegypti* paired groups of each species were fed on a series of different chicks with varying degrees of infection. It is evident from the data summarized in table 1 that *A. quadrimaculatus* is significantly more refractory to infection by *P. gallinaceum* that is *A. aegypti*. In this series, only

TABLE 1.—*A comparison of the rate of infection and the degree of infection of laboratory strains of* A. aegypti *and* A. quadrimaculatus *simultaneously infected with* P. gallinaceum.

	Number of oöcysts per mosquito					Total mosquitoes	Number infected	Percent infected
	0	1–99	100–299	300–499	500+			
	Number of mosquitoes							
A. aegypti	8	188	63	8	2	269	261	97.0
A. quadrimaculatus	163	69	15	5	12	264	101	38.3

Again, even though opposite in effect, folic acid, like the sulfonamides, paraaminobenzoic acid, and the antibiotics, has no further effect on the host-parasite equilibrium at concentrations sufficiently above the critical levels. At a concentration of 0.05% the effect of the drug on host resistance is essentially eliminated, and there is no significant difference in distribution according to oöcyst density between the mosquitoes in control group 174-23 and the individuals in experimental group 174-24. Thus, in its concentration-action behavior, folic acid, which is able to increase host resistance in this host-parasite relation, acts in a manner similar to the compounds which increase host susceptibility.

To provide evidence that this specific effect of antibiotic or metabolite on susceptibility or resistance is fundamental to the mechanism of innate immunity in host-parasite relations in general, rather than an isolated phenomenon unique to a single host-parasite combination, 38.3% of the *Anophelines* developed infections whereas 97.0% of the *Aëdes* became parasitized.

It is of interest to note that even though *A. quadrimaculatus* is much more resistant to infection by *P. gallinaceum* than is *A. aegypti*, nevertheless among the individuals that do become infected there are a significant number that develop infections far more intense than the infections in the *A. aegypti*. For example, among the 264 *A. quadrimaculatus* dissected, there were 12 individuals with counts above 500 oöcysts, and of these, seven had counts well above 600 oöcysts, while three had counts estimated to be above 900 oöcysts. On the other hand, among the 269 *A. aegypti* dissected, there were only two mosquitoes with counts above 500, and the highest count recorded was 520 oöcysts. Thus, there occur among the more resistant *A. quadrimaculatus* a significant number of individuals whose susceptibility to infection is even greater than that of the generally more suscep-

tible *A. aegypti*. It may be noted, too, that even in a susceptible species like *A. aegypti* there is a well defined resistance to infection by *P. gallinaceum*. From determinations of the amount of blood taken up by the mosquito during an infective feed, gametocyte counts of the mosquito stomach contents after feeding, and blood counts and gametocyte counts of the chick used as the source of infection, it has been possible to establish with reasonable accuracy the parasite numbers in the inoculum taken up by the mosquito. From these data and from a subsequent determination of the oöcyst counts of the mosquitoes infected with a known parasite inoculum, it has been calculated that in general considerably less than 1% of the parasites succeed in becoming established in the host mosquito. As the experiments in this study show, this parasite mortality must be due in a large measure to the immune mechanisms of the host rather than to a lack of viability on the part of the parasite.

The same techniques and methods used with *A. aegypti* and *P. gallinaceum* were used with *A. quadrimaculatus* and *P. gallinaceum*, and similarly, each experimental group was paired with a control group and both were fed simultaneously on the same infected chick. However, because chicks do not withstand feeding by *A. quadrimaculatus* as well as they withstand feeding by *A. aegypti*, it was not usually possible to infect an entire experimental series on a single animal. Furthermore, because so many of the *A. quadrimaculatus* fail to develop infections, much larger numbers of this species had to be used, and it was therefore necessary to combine the results of different series of experiments. The data presented in each of the following tables represent a summary of the results of 10 to 15 series of experiments with each of the compounds studied.

TABLE 2.—*A comparison of the rate and degree of infection of control groups of* A. quadrimaculatus *maintained on 4% sugar and infected with* P. gallinaceum *and of simultaneously infected experimental groups maintained on 4% sugar with various concentrations of para-aminobenzoic acid added.*

	Number of oöcysts per mosquito					Total mosquitoes	Number infected	Percent infected	*P* values for differences between groups	
	0	1–99	100–299	300–499	500+				Number infected	Degree of infection
	Number of mosquitoes									
Control	114	57	14	5	1	191	77	40.3	0.20	0.80
0.01 PABA	113	82	15	5	4	219	106	48.4		
Control	186	93	22	7	2	310	124	40.0	<0.01	<0.01
0.1 PABA	139	99	26	16	19	299	160	53.5		
Control	182	86	20	6	7	301	119	39.5	<0.01	<0.01
0.2 PABA	151	89	28	14	33	315	164	52.1		
Control	177	71	12	6	1	267	90	33.7	0.90	0.90
0.4 PABA	164	63	13	7	3	250	86	34.4		

The data presented in table 2 show that para-aminobenzoic acid, over a wide range of concentrations, has increased the innate susceptibility of *A. quadrimaculatus* to infection by *P. gallinaceum* to an extent such that significantly greater numbers of the mosquitoes maintained on this metabolite developed infections, in comparison to the individuals maintained on sugar alone. For example, 48.4% of the mosquitoes maintained on 0.01% para-aminobenzoic acid developed infections, while only 40.3% of the control animals became infected. At higher concentrations

the differences become even more pronounced, so that whereas 53.5% of the mosquitoes maintained on 0.1% para-aminobenzoic acid and 52.1% of those maintained on 0.2% para-aminobenzoic acid became infected, only 40.0% of one control group and 39.5% of the other control group developed infections. On the other hand, in concentrations above these levels para-aminobenzoic acid had no discernible effect on the host-parasite equilibrium, and while oöcysts and only nine individuals with counts above 500 oöcysts. Furthermore, among the mosquitoes maintained on 0.1 and 0.2% para-aminobenzoic acid the counts ranged above an estimated 1200 oöcysts, whereas the highest count recorded among the control mosquitoes was an estimated 900 oöcysts. On the other hand, in the group maintained on 0.01% para-aminobenzoic acid, even though the infection rate was higher than that of the controls, nevertheless

TABLE 3.—*A comparison of the rate and degree of infection of control groups of* A. quadrimaculatus *infected with* P. gallinaceum *and similarly infected experimental groups maintained on sulfadiazine.*

	Number of oöcysts per mosquito					Total mosquitoes	Number infected	Percent infected	*P* values for differences between groups	
	0	1–99	100–299	300–499	500+				Number infected	Degree of infection
	Number of mosquitoes									
Control	192	109	19	8	4	332	140	42.2	0.75	0.60
0.001 Sulfa	198	98	24	10	3	333	135	40.5		
Control	188	109	14	5	1	317	129	40.7	0.40	0.80
0.01 Sulfa	211	96	16	5	1	329	118	35.9		
Control	176	136	24	10	3	349	173	49.6	<0.01	0.80
0.1 Sulfa	290	50	8	3		351	61	17.4		
Control	151	96	24	8	1	280	128	45.7	<0.01	<0.05
0.2 Sulfa	239	33	2	1		275	36	13.1		

34.4% of the mosquitoes maintained on 0.4% para-aminobenzoic acid developed infections, the infection rate remained essentially the same in the controls in which 33.7% of the individuals became parasitized.

In addition to increasing the susceptibility of *A. quadrimaculatus* to *P. gallinaceum* to an extent whereby more individuals in a given population become infected, para-aminobenzoic acid also effects an increase in the degree of infection so that among the infected mosquitoes greater numbers develop significantly heavier infections. Thus, in the two experimental groups maintained on 0.1% and 0.2% para-aminobenzoic acid, there were 30 mosquitoes with counts above 300 oöcysts and 52 mosquitoes with counts above 500 oöcysts, while in the controls of these groups there were 13 mosquitoes with counts above 300 the relative distribution according to oöcyst density was approximately the same in both groups. And again, a concentration of 0.4% para-aminobenzoic acid showed no effect on host susceptibility, and the frequency distribution according to oöcyst density was the same in both experimental and control groups.

It has been shown that in the host-parasite relation between *A. quadrimaculatus* and *P. gallinaceum* para-aminobenzoic acid increases host susceptibility in a manner similar to its behavior in the relation between *A. aegypti* and *P. gallinaceum*. It is important to note, therefore, that sodium sulfadiazine, on the other hand, reverses its effect in *A. quadrimaculatus* and is characterized instead by an ability to increase host resistance to *P. gallinaceum*.

The data presented in table 3 show

that at 0.001% and 0.01% concentrations of sulfadiazine there is no evident decrease in the rate of infection in the experimental groups as compared to their respective control goups. With the concentration of sulfadiazine increased to 0.1% and 0.2%, however, the drug produces a striking increase in host resistance, evidenced by the significantly lower infection rates in the drug treated groups. Thus, whereas only 17.4% of the mosquitoes maintained on 0.1% sulfadiazine and 13.1% of the mosquitoes maintained on 0.2% sulfadiazine became infected, 49.6% of the mosquitoes in the one control group and 45.7% of the individuals in the other control group developed infections. This effect on host resistance was further evidenced by the fact that the drug-fed mosquitoes developed extremely light infections as compared to the level of infection in the respective control mosquitoes. Consequently, while there were only 14 mosquitoes with counts above 100 oöcysts in the groups maintained on 0.1 and 0.2% sulfadiazine, there were 70 mosquitoes with oöcyst counts above this figure in the control groups maintained on sugar. Finally, it is of interest to note that in *A. quadrimaculatus* infected with *P. gallinaceum*, unlike its concentration-action in behavior in *A. aegypti* infected with *P. gallinaceum*, sulfadiazine showed no diminution in its activity with increasing concentration. In fact, as reference to table 3 shows, host resistance to infection was maximal at maximum tolerated doses so that the least numbers of mosquitoes became infected in the groups maintained on 0.2% concentrations of sulfadiazine and these mosquitoes had the lightest infections of any in the series.

Like sulfadiazine, aureomycin also reverses its effect in *A. quadrimaculatus* and produces instead an increased host resistance to *P. gallinaceum*. At a concentration of 0.001% aureomycin has little effect, and there is no significant decrease in the rate of infection of the experimental groups as compared to the control groups. At a concentration of 0.005%, however, aureomycin has a marked effect, and host resistance is increased to the extent that only 35.8% of the experimental animals developed infections as compared to 49.5% of the control mosquitoes. With concentration increased to 0.01% aureomycin produced a further striking increase in host resistance to infection, so that only 19.8% of the drug-fed mosquitoes became parasitized, whereas 48.8% of the control mosquitoes maintained on sugar developed infections. And again, the effect on host resistance was further evidenced by the lower oöcyst densities in the mosquitoes maintained on aureomycin as compared to the mosquitoes maintained on sugar alone.

On the other hand, like para-amino-

TABLE 4.—*A comparison of the rate and degree of infection of control groups of* A. quadrimaculatus *infected with* P. gallinaceum *and similarly infected experimental groups maintained on folic acid.*

	Number of oöcytes per mosquito					Total mosquitoes	Number infected	Percent infected	*P* values for differences between groups	
	0	1–99	100–299	300–499	500+				Number infected	Degree of infection
	Number of mosquitoes									
Control	137	80	20	4	2	243	106	43.6	0.20	0.50
0.01 PGA	143	60	15	7	3	228	85	37.3		
Control	208	90	23	9	5	335	127	37.9	<0.01	<0.01
0.1 PGA	250	69	16	1	1	337	87	25.8		
Control	153	87	27	15	3	285	132	46.3	<0.01	<0.01
1.0 PGA	231	51	6			288	57	19.8		
Control	143	74	23	12	4	256	113	44.1	<0.01	<0.01
2.0 PGA	225	28	1	3	1	258	33	12.8		

benzoic acid, folic acid does not reverse its effect in a new host but, as shown in table 4, produces the same increase in host resistance in *A. quadrimaculatus* infected with *P. gallinaceum* as it did in *A. aegypti* infected with this parasite. Thus, administered in a concentration of 0.001%, folic acid begins to show evidence of increasing host resistance to infection. With increasing concentration there is a corresponding increase in host resistance so that at maximum tolerated doses folic acid produces its maximum effect on the host-parasite equilibrium. The infection rate in the mosquitoes maintained on 0.1% folic acid was 12.1% lower than the infection rate of the control mosquitoes, 26.5% lower in the mosquitoes maintained on 1.0% folic acid, and finally, 31.3% lower in the mosquitoes maintained on 2.0% folic acid than in the respective control mosquitoes. And again, the increase in host resistance was further reflected in the lower parasitemias in the mosquitoes maintained on folic acid as compared to the control mosquitoes maintained on sugar. There were only 11 mosquitoes with counts above 100 oöcysts in the groups administered 1.0% and 2.0% folic acid, whereas there were a total of 84 mosquitoes with more than 100 oöcysts in the two control groups. Finally, it may be noted that even though folic acid does not reverse its effect in the new host, nevertheless, in its concentration-action relations it does not behave in *A. quadrimaculatus* as it does in *A. aegypti*. Unlike its activity in *A. aegypti*, in which host resistance was not affected above critical concentrations, it has been shown that in *A. quadrimaculatus* the activity of folic acid continued to increase with increasing concentration and it produced its maximum effect at maximum tolerated doses.

DISCUSSION

It has been impossible thus far to design and execute an experiment that can show unequivocally whether the effects of these compounds on the host-parasite relation are directly on the host or on the parasite. However, a consideration of the behavior and the characteristics of these drugs and metabolites in this host-parasite relation indicates that they produce their effects essentially on the host rather than on the parasite. In the first place, it has already been shown that sulfadiazine inhibits the development of *P. gallinaceum* in *A. aegypti*.[6] It appears unlikely, therefore, that a compound which can at one time arrest the development of the parasite could at another stage enhance the virulence or invasive powers of the same parasite. Secondly, sulfadiazine produces the same inhibitory effect on the parasite in *A. quadrimaculatus* as it does in *A. aegypti*, so that insofar as effect on the parasite is concerned metabolism of the drug is the same in both hosts. Nevertheless, in *A. quadrimaculatus* sulfadiazine reverses the effect on host immunity that it produced in *A. aegypti* and in this case produces instead an increased host resistance to infection with *P. gallinaceum*. Like sulfadiazine, aureomycin, which has a similar inhibitory effect on *P. gallinaceum* in either host, increases susceptibility to *P. gallinaceum* in *A. aegypti* and then reverses its action and increases resistance to this parasite in *A. quadrimaculatus*.

Furthermore, although sulfadiazine and aureomycin, which are able to produce changes in host resistance or susceptibility, have a specific inhibitory effect on the parasite, nevertheless, streptomycin, para-aminobenzoic acid, and folic acid, which are similarly able to affect host immunity, have no discernible

6. Terzian, L. A., Stahler, N. and Weathersby, A. B. 1949, The action of antimalarial drugs in mosquitoes infected with *Plasmodium gallinaceum*. J. Infect. Dis. **84**: 47–55.

effect whatsoever on the parasite, neither inhibiting nor accelerating any stage of the parasite developmental cycle. The ability of these compounds to produce changes in host reaction to infection, therefore, is completely unrelated to any possible effects on the parasite. Thus, there is ample reason to assume that these compounds produce their specific effects by interfering, in some manner not yet understood but related perhaps to hormonal balance, with the innate immunity mechanism of the host, and thus affecting the inherent balance in this host-parasite relation.

It is important to note, too, that the sulfonamides, the antibiotics, and the metabolites have a specific similarity of action and effect in their ability to produce changes in host susceptibility or resistance to infection. On the other hand, in spite of the similarity in effect the evidence thus far indicates that each compound acts through an independent mechanism, the nature of which has not yet been characterized.

It is of interest to note, too, that in producing these effects on host immunity, the sulfonamides and antibiotics appear to have greater potentialities of action than do the metabolites. Thus, in either host, para-aminobenzoic acid was able only to increase susceptibility while folic acid consistently produced an increase in host resistance. On the other hand, sulfadiazine and aureomycin, both of which had increased the susceptibility of *A. aegypti*, were able to reverse the qualitative effects of their action and to increase the resistance of *A. quadrimaculatus*.

Finally, it is evident that these compounds have a fundamental relation to the mechanism of innate immunity and host-parasite specificity, a relation which may obtain with other similar compounds and other host-parasite relations.

CONCLUSIONS

1. It has been demonstrated quantitatively that specific biochemical compounds can influence the innate immunity of a host to a parasite. Thus, it has been shown that in *Aëdes aegypti* sulfadiazine, para-aminobenzoic acid, aureomycin, and streptomycin, administered in critical concentrations, increase host susceptibility to infection with *Plasmodium gallinaceum*, whereas folic acid increases host resistance to infection with this parasite. In addition, it has been shown that in *Anopheles quadrimaculatus*, again para-aminobenzoic acid increases host susceptibility to *P. gallinaceum* and folic acid increases host resistance to this parasite, but that sulfadiazine and aureomycin, on the other hand, reverse their effects and both increase host resistance to infection.

2. In *A. aegypti*, the maximum effects of sulfadiazine, para-aminobenzoic acid, folic acid, aureomycin, and streptomycin on the host-parasite equilibrium depend upon a specific optimal range of drug concentrations above which the specific effects of these compounds on susceptibility or resistance are depressed or eliminated. In *A. quadrimaculatus*, only para-aminobenzoic acid continues to show this concentration-action relation, whereas sulfadiazine, aureomycin, and folic acid produce their maximum effects at maximum tolerated doses.

3. It is evident that the sulfonamides, the antibiotics aureomycin and streptomycin, and the metabolites para-aminobenzoic acid and folic acid basically resemble one another in their physiological activity and in their ability to produce similar effects on host susceptibility or resistance to infection, even though they may produce these effects through different mechanisms. And finally, it is evident that these compounds have, in addition, a fundamental relation to the mechanisms of innate immunity and host-parasite specificity.

SYLVATIC PLAGUE STUDIES

VII. PLAGUE TRANSMISSION POTENTIALS OF THE FLEAS *DIAMANUS MONTANUS* AND *POLYGENIS GWYNI* COMPARED WITH *XENOPSYLLA CHEOPIS*

R. HOLDENRIED

From the George Williams Hooper Foundation, University of California, San Francisco, California

In the western United States sylvatic plague is distributed over a wide geographic area, and by June, 1950, *Pasteurella pestis* (Lehmann and Neumann, 1896) had been isolated from the tissues of 31 species of native wild rodents. One of the most frequent victims of plague in this area—*Citellus beecheyi* (Richardson, 1829), the California ground squirrel—was the first animal to be studied by epidemiologists and ecologists, and the plague transmission ability of its fleas has been investigated in the laboratory. One of these fleas, *Diamanus montanus* (Baker, 1895), has been considered a vector of paramount importance, but reports of its vector efficiency conflict.

Burroughs (1947) offered a theoretically plausible explanation for the reported differences: that different populations of *D. montanus* from widely separated geographic areas may transmit plague at different rates. The principal object of the present study was to investigate whether the transmission potential of this flea varied in selected, widely separated areas in central California.

In addition, the plague transmission of *Polygenis gwyni* (Fox, 1914), a cotton rat flea regularly infesting both cotton rats (*Sigmodon hispidus*, Say and Ord, 1825) and Norway rats (*Rattus norvegicus*, Erxleben, 1777) in the south and southwestern United States, was also investigated in an attempt to learn whether it could transmit plague among rodents. In areas inhabited both by infected cotton rats and susceptible Norway rats, plague might be transferred to the domestic rats by these fleas.

Xenopsylla cheopis (Rothschild, 1903) was used to check the procedures before the primary experiments were begun and as a control, since its high vector efficiency is undisputed.

D. montanus was first demonstrated to be capable of plague transmission by McCoy (1910). In one experiment 89 fleas placed on a laboratory-infected ground squirrel later transmitted the infection to two of four guinea pigs; in another experiment 25 fleas removed from a dead laboratory-infected ground squirrel infected a second healthy ground squirrel. In Eskey and Haas's study in 1941, 19 fleas were infected with plague. These were fed individually on healthy guinea pigs and two transmitted the infection. In their effort to precisely evaluate the plague vector efficiency of different species of fleas, Wheeler and Douglas (1941) originally assigned to *D. montanus* a vector effi-

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The present study was part of the George Williams Hooper Foundation's investigations on plague and was carried on with the advice and guidance of Dr. K. F. Meyer. Professors M. A. Stewart and D. P. Furman of the University of California offered additional supervision. On various occasions, Mr. A. H. Dahl, Chief of the Bureau of Vector Control, California State Department of Public Health, delegated a Vector Survey Crew to aid in the field collection of fleas.

TABLE 1.—*Source of the fleas.*

Species	Place	Elevation, feet	Year collected	Plague history*
D. montanus	1½ to 3 miles east of Castaic Lake (near Lebec), Kern County, Calif.	4000	1948	1941; 6 of 7 yr. 1941–1946
	St. John, Glenn County, Sacramento Valley, Calif.	150	1948	None
	Near Napa-Sonoma County Line, 4 miles east of Schellville on Highway 37, Calif.	100	1948	None
	Calaveras Dam, Alameda County, Calif.	700	1948	1933
				1942
	Santa Margarita, San Luis Obispo County, Calif.	1000	1949	1924
				1925
				1946
				1947
				1948
	San Joaquin Experimental Range, O'Neals, Madera County, Calif.	1000	1948	None
			1949	
P. gwyni	Grady and Brooks Counties, Georgia		1948	None
X. cheopis	San Francisco		1944	Many years

* *Past. pestis* found in rodents or fleas.

ciency of 1.14,* and to *X. cheopis* one of 0.39, but more extensive testing of these same species later gave slightly different results (Wheeler and Douglas, 1945): 0.84 and 0.43, respectively, but *D. montanus* was still nearly twice as efficient as *X. cheopis*. In contrast, in another study (Burroughs, 1947) *D. montanus* was a very poor transmitter, while the vector efficiency of *X. cheopis* corresponded to that given in the former studies.

MATERIALS AND METHODS

Fleas.—Details about the sources of the fleas are given in table 1. The *D. montanus* fleas were collected from ground squirrels, and in all but two instances these field-collected fleas formed a breeding nucleus for a laboratory colony used for transmission experiments. In the two excepted instances the field-collected fleas were used directly in experiments within a few days after they were brought to the laboratory. All of the localities from which *D. montanus* were obtained had been surveyed for plague by the California State Department of Public Health. The existence of sylvatic plague in the areas where the fleas were collected was proved in two, but there was no evidence that plague was epizootic. The Calaveras Dam area was the source of the *D. montanus* tested by Burroughs (1947) and the San Joaquin Experimental Range the source of those used by Wheeler and Douglas (1945).

The stock of *X. cheopis* (the same as that used by Burroughs, 1947) had been maintained continuously in the plague laboratory of the Hooper Foundation for approximately five years. The original stock was collected from rats in San Francisco.

Strains of Past. pestis.—The Shasta strain of *Past. pestis* (isolated from a human infection in the city of Mount Shasta, California in 1941) was used in all except one experiment because it is stable and highly virulent. Burroughs (1947) also used this strain in his flea transmission work. In a single experiment, SB258 (originally isolated from ground squirrels from San Benito County, California in 1946) was used. The strain used by Douglas and Wheeler (1943) was isolated from a naturally infected ground squirrel and it seemed advisable in attempting to repeat their work to used a plague strain of similar origin.

The plague strains were maintained on blood-agar medium between passages through laboratory mice and guinea pigs. For preparation of an inoculum, the organisms are grown for 24 hours in hormone beef-broth; then a portion of this culture is transferred to a new beef-broth medium. This second culture is diluted after 24 hours to 10^{-3} in normal saline, and 0.2 ml (containing from 20,000 to 40,000 organisms) are inoculated intraperitoneally into white mice. Usually the mice died within 40 to 50 hours with intense bacteremia.

Flea-rearing in the laboratory.—Fleas were reared by the method described by Douglas and Wheeler (1943) and by Burroughs (1947). A cotton rat served as the host for *P. gwyni*. All other fleas were cultured with white rats as hosts.

Infection and examination of fleas.—As white mice previously inoculated with *Past. pestis* appeared to be close to death, blood smears were made. When the ratio of plague bacilli to erythrocytes was approximately 1:5, fleas were placed on the mouse. Mice with such an intense bacteremia usually died in from 15 to 30 minutes and

* The vector efficiency is the quotient of the total number of transmissions by fleas given only one daily opportunity to feed on mice, divided by the total number of fleas used in the experiment.

the fleas were removed promptly from the dead hosts. Each flea was examined under the microscope to make sure that it had a bright red ventriculus, indicating that it had ingested infected blood. In order to make the examination, the flea was placed between a cover slip and glass. The ingested blood and other ventricular contents surged about because of the peristaltic action of the ventriculus. The pumping action of the pharyngeal pump, eggs in various stages of development, salivary glands, fat bodies, malpighian tubules, trachea and muscles could also be observed.

Feeding and holding infected fleas.—Usually immediately after the fleas had ingested blood, they were separated by microscopic examination according to sex and as many as 100 were held for from one to three days at from 65 to 72 F in a 250-ml jar with a fairly tight-fitting cap, containing enough wood shavings to cover the bottom.

After this one- to three-day fast, the vector ability of the flea was tested. One of two methods of feeding infected fleas separately was used. They were either left on the mouse to feed at will, or they were retained in vials and fed at definite intervals. In the former instance, mice were held singly for one to 43 days in one-quart jars containing a thin layer of sawdust and capped with a wire mesh cover. They were not given water, but a cube of carrot was provided, along with their usual diet of rolled oats, three times a week. The jars were retained in five-gallon crocks to prevent escape of any fleas that might crawl out of the jars.

X. cheopis frequently remained on the mouse near the base of its tail; *D. montanus* rested in the sawdust in the bottom of the jar rather than on the mouse. Mice occasionally removed and killed the fleas by biting. *X. cheopis* and *D. montanus* were killed in this way infrequently, but *P. gwyni* apparently irritated the mouse and was killed, as a rule, within a few minutes after being placed on it.

These conditions simulated the natural ones more closely than did those provided in glass tubes (Verjbitzki, 1908; Eskey and Haas, 1940; Douglas and Wheeler, 1943; Burroughs, 1947) because adult fleas could be left with the hosts for for days and feed at will. The possibility of transmitting plague is also increased. Transmission by a flea whose alimentary tract is blocked by a plug containing *Past. pestis* is more likely to follow prolonged efforts to feed than shorter attempts (Eskey and Haas, 1940; Burroughs, 1947).

By the second method (Douglas and Wheeler, 1943; Burroughs, 1947), each flea was placed in a separate small glass screw-cap shell vial with a metal cap. A perforation in the cap was covered by no. 72 grit silk gauze. These vials were stored at 70 F in a desiccator over a saturated solution of potassium dichromate (relative humidity at 70 F = 50%).

When a flea was to be fed on a normal mouse, the metal cap was removed and the vial and mouse were placed in a holder (described by Burroughs, 1947). The flea was allowed to feed for from ½ to 1½ hours once a day from one to nine days on the clipped belly of the same mouse. Mice were observed for at least 21 days after the last exposure. Transmission was recorded only if *Past. pestis* was cultured from heart blood of the dead mouse.

The white mice used were of various strains and diverse ages. Mouse resistance, if present, did not influence the results, as is evident in the results obtained with control experiments using the same stock of mice. Limitations of time and laboratory space prohibited supplying a new mouse to each flea every day, but mice were changed often enough so that it is known that transmission occurred within a period of a few days. The object of the experiments was to determine whether, not when, *Past. pestis* was transmitted.

Experiments were either terminated after a definite number of days, usually 21, or were continued until all fleas died, depending on the conditions of the individual experiment. Previous plague transmission experiments indicated that *X. cheopis* and *D. montanus* rarely transmit after more than 21 days after the infectious meal (a meal of plague-infected blood), and usually few of these fleas survive longer than this time. Fleas continued to die and as a plague-transmission experiment progressed, the number of surviving fleas continually decreased.

To determine the proportion of the experimentally infected fleas that retained the infection at death or at the termination of an experiment, fleas were often triturated, diluted with two or three drops of normal saline and inoculated into mice or plated on blood-agar medium or on a plague selective medium described by Meyer and Batchelder (1926).

For each strain of fleas, the number tested for plague transmission ability is given. This number is somewhat arbitrary and represents the number of fleas that lived for at least five days after the infectious meal, because only fleas living these five or more days were capable of transmitting the infection.

The number of days that a plague-infected flea remained alive indicates to a certain extent the duration of its opportunity to transmit the infection. The great majority of the plague infec-

TABLE 2.—*Survival of fleas after plague-infectious meals.*

Days after meals	Survivors*				
	Sex			Total	Percent
	Males	Females	Not known		
	D. montanus				
5	174	272		446	100
10	127	199		326	74
15	104	156		260	58
20	60	99		159†	36
	P. gwyni				
5	39	49		88	100
10	23	40		63	79
15	14	24		38	43
20	9	14		23	26
25	7	9		16‡	18
	X. cheopis				
5	121	137	27	285	100
10	97	113	24	234	82
15	76	84	21	181	62
20	47	55	15	117§	41

* Only fleas surviving at least 5 days after the infectious meal were used.

† By the 25th day, 56 had died and 16 were killed; 87 remained alive. Of these, 48 were alive on the 30th day, 32 having died and 7 having been killed. By the 35th day, 17 remained alive, 4 had died and 27 were killed. Twelve were alive on the 40th day, 5 more having died. On the 45th day, 5 remained alive, 4 had been killed. Three were alive on the 50th day and 2 on the 55th day. All were dead on the 60th day.

‡ One of these fleas was killed on the 26th day. Nine, 6 and 5 fleas remained alive on the 30th, 35th and 40th days, respectively. On the 51st day the remaining 5 were killed.

§ After the 21st day, experiments were terminated irregularly, frequently with the killing of the surviving fleas. There was a reduction of 49 fleas between the 20th and the 25th days; 33 of these were killed. By the 30th day, 41 remained—12 had died and 15 were killed in the preceding 5 days. On the 31st day, 21 *X. cheopis* were killed; 3 remained alive on the 35th day. Two of these had died by the 40th day; the last flea died on the 42nd day.

tive fleas retained at temperatures of 68 to 72 F or higher transmitted before the end of the third week. On the average, they transmit *Past. pestis* in the second week. This period between the infectious meal and the time that the flea could transmit plague—the extrinsic incubation period—is modified by the number and frequency of blood meals after the infectious blood meal, as well as by the temperature to which this flea is exposed and by other unknown factors. Many potentially infective fleas die without transmitting plague, and those that do transmit usually die within three or four days after their first transmission. Obviously, to evaluate properly the experimental plague vector efficiencies of flea species, the survival rate under the conditions of the experiment is of importance and may significantly influence the results. In the present study the survival of each species of flea used in the experiments at five-day intervals is given in table 2.

RESULTS

D. montanus

Results of experiments with this species are given in table 3.

These strains were obtained both from known sylvatic plague foci and from areas from which plague has never been reported. Three strains from the plague foci of Castaic Lake, Calaveras Dam and Santa Margarita had such low transmission potentials that plague was not transmitted once by the fleas tested. The strain originating from the Sonoma-Napa County line area, a region from which plague has not been reported, did not transmit. The strains from St. John in the Sacramento Valley and from the San Joaquin Experimental Range in the San Joaquin Valley both

TABLE 3.—*Summary of* D. montanus *plague transmission experiments.*

Flea strain	Number		Feeding procedure*	Fleas transmitting		Controls†	
	Laboratory-reared	Field-collected		Number	Percent	Total	Transmitting
Castaic Lake	94		1	0	0	12	4
Calaveras Dam	53		1	0	0	11	3
St. John	61		1	3	5	30	21
Napa	51		1	0	0		
Santa Margarita	18		1	0	0	22	18
		21	2	0	0		
San Joaquin	38		1	2	5	20	17
Experimental	31		3	4	13	10	4
Range	28		4	0	0	12	11
	12		5	0	0		
		39	4	0	0	12	8
Total	386	60		9	2		

* 1 = Free on mice continuously.
2 = Free on mice Tuesday, Thursday, Friday and Saturday of each week.
3 = Free on mice on 3 nonconsecutive days each week.
4 = Vials, daily for 19 days and then 6 times a week.
5 = Free on mice daily for 3 to 5 hours for 19 days, then 6 times a week.

† The Control *X. cheopis* are not totaled because single groups at times served as controls for more than one strain of *D. montanus*. This group of fleas will be discussed in greater detail in another section of this paper.

transmitted plague to mice. So far as is known, enzootic sylvatic plague has not occurred in the Sacramento Valley nor on the San Joaquin Experimental Range. Three of the 61 St. John fleas transmitted plague. Several changes in the procedures in the transmission experiments failed to produce a combination of conditions favorable for transmission by the 148 fleas of the San Joaquin Experimental Range strain and only six transmitted plague.

A total of 446 *D. montanus* in the six strains were infected and tested and of these only nine (2%) transmitted plague. This indicates that this species has a very low vector potential.

Among those strains tested, the difference in the plague transmission potential, while apparent, was of such low magnitude that the significance must be questioned. Only by testing considerably larger numbers of individuals in all of the strains could truly comparative values be obtained.

TABLE 4.—*Summary of* P. gwyni *plague transmission experiments.*

Exp. no.	Total fleas		Transmitters			Controls (*X. cheopis*)	
	Males	Females	Males	Females	Percent	Number	Transmitters
1	24	33	6	10	28	11	8
2	15	16	5	3	26	10	5
Totals	39	49	11	13	27*	21	13

* Fourteen fleas transmitted to 1 mouse each, 7 transmitted to 2 mice each and 3 transmitted to 3 mice each.

P. gwyni

A total of 88 infected cotton rat fleas, *P. gwyni*, were tested for plague transmission ability in two experiments, 57 (24 males, 33 females) in the first and 31 (15 males, 16 females) in the second (table 4). In the first (exp. 1), 53 fleas were held individually in vials and given opportunities to feed six days a week for 7½ weeks. The first opportunity to feed on healthy mice was provided the day after the fleas had fed on an infected mouse. At the termination of the experiment on the 51st day after the infectious blood meal, the five fleas remaining alive were given their last opportunity to feed. Each of four additional females was put into a jar with a mouse two days after the infectious blood meal. In preliminary work, when this species of flea was placed free on mice, the fleas were frequently bitten and killed. By limiting the time that the flea and mouse were together, the mouse had fewer opportunities to bite the flea and the survival chances for the fleas were increased. The mice were removed from the fleas every other day and returned 24 hours later, except over the weekend, when they remained away from the fleas for two days. The last of these four fleas died 19 days after it had ingested infected blood.

To determine whether the results of the plague transmission tests obtained with the first group of *P. gwyni* were reproducible, the second group (exp. 2) was tested for plague transmission. Plague had been transmitted in only one instance in the first group as early as the third day after the infectious blood meal. For this reason, individual flea feedings in the second group were omitted until the fourth day after the infectious blood meal. In the second group the males and females were held in separate quart jars so that there would be no more sexual activity than in the first group held in vials. For the first three days after the infectious meal, a healthy mouse muzzled with a piece of adhesive tape ¼-inch wide to prevent it from biting the fleas was introduced into each jar daily. The mouse was

left with fleas for up to two hours. Beginning with the fourth day, these fleas were placed individually in vials where they remained until the end of the experiment. Each was given an opportunity to feed on a white mouse daily for the next four days, then starved for four days and then fed again daily for four days. After two more days of fasting, the fleas were again given opportunities to obtain blood from mice on each of the next six days. The experiment terminated on the 26th day with the killing of the single surviving flea. The survival rate of the fleas in both groups is shown in table 2.

The two groups of fleas were given a total of 932 individual opportunities to feed. After 244 (26%) of these opportunities, the fleas had blood in the esophagus, but because of blockage in the region of the proventriculus, they could not force blood into the ventriculus. In addition, on 12 occasions, a continuous column of blood extended from the ventriculus into the esophagus. Apparently the function of the proventricular valve was impaired by packing of plague bacilli among the spines, as described by Bacot (1915).

In these *P. gwyni*, complete blockage of the alimentary tract by plugs containing *Past. pestis* was seen very frequently—in 37 (76%) of 49 females and in 22 (56%) of 39 males, or a total of 59 (67%) of 88 fleas. Probably additional individual fleas had such occlusions, but they could not be detected because the fleas did not attempt to feed and hence there was no diagnostic column of fresh red blood in the esophagus, ending posteriorly abruptly at the obstruction.

The 82 fleas that died during the experiments were cultured bacteriologically for the presence of viable plague organisms; of these, 68 (83%) were positive. The five fleas killed on the 51st day were negative.

P. gwyni can remain alive under laboratory conditions for many days after the alimentary tract has become obstructed. In the first experiment, the average period of life after the alimentary canal became plugged was ten days. In the second, opportunities to feed were greatly reduced and observations were fewer. However, the average period between the first day of occlusion and the death of the flea was six days. The average for both groups was nine days. The period was the longest in three female *P. gwyni* who lived 29, 29 and 26 days, respectively, after the alimentary tract obstruction developed. However, in this period all three fleas forced blood past the obstruction and fed successfully at least once. Male fleas also lived for some time after their alimentary canals were completely occluded. For example, a male flea lived 15 days after its alimentary canal became occluded and blood could not reach its ventriculus. This flea fed once and died ten days later without again being able to force blood into its ventriculus.

As mentioned earlier, a procedure of allowing each flea repeated opportunities to feed on a single mouse was followed. Single *P. gwyni* were given opportunities to feed daily on single mice for as long as six days. The average extrinsic incubation period in the present *P. gwyni* plague transmission experiments therefore fell in a three-day period of from nine to 11 days, inclusive, after the fleas had fed on an infected mouse. Between 15 and 21% of the transmissions occurred during the first week; one female flea had transmitted plague by the second day. All but five (13%) of the 37 transmissions were accomplished by the 15th day. A male flea had the longest extrinsic incubation

period, not transmitting until the 23rd or 24th day.

X. cheopis

This flea served as a control in the transmission experiments with other species and was also used to check the survival and transmission rate of the plague-infected individual fleas continuously free on mice. In the following discussion, the former group will be designated as control *X. cheopis* and the latter group as "Experiment A."

Experiment A with fleas free on mice was performed with 155 infected *X. cheopis* starved for three days after infection. After three weeks the rate of plague transmissions was greatly reduced, and to determine whether the fleas surviving contained *Past. pestis*, they were killed at intervals, individually triturated and tested bacteriologically by inoculation into healthy mice or by culturing on blood-agar plates. Twenty-four days after their meal of infected blood, 13 of these fleas were killed; seven retained *Past. pestis*. Sixteen more fleas were killed and examined bacteriologically on the 25th day; 15 contained the organisms. Nine fleas were killed 28 days after the infected blood meal; three harbored *Past pestis*. The remaining ten fleas were killed on the 31st day; five were positive. In all, then, 48 fleas were killed and 30 (62%) of these were infected with *Past. pestis*. Fifty-two other fleas that died during the plague transmission experiments were also examined bacteriologically; plague organisms were recovered from 33 (63%). The survival rate of the fleas in this experiment is shown in table 2.

Since in this plague transmission experiment the fleas were disturbed as little as possible, they were examined microscopically only to insure that they had fed on the infected mouse. After they were placed on the healthy mice in jars, they were not examined again until they died or were killed. For this reason, detailed information about blocking of the alimentary tract was not obtained.

For controls in the *D. montanus* experiments, 130 *X. cheopis* were used. This total is not the sum of the controls used in each experiment because at times the same group of *X. cheopis* served as controls for two or more experiments conducted simultaneously.

The survival rate of the control and experiment A *X. cheopis* is summarized in table 2. Whether they were killed after 21 days or at a later date depended on the procedure of the experiment in which they served as controls.

To determine if the fleas retained *Past. pestis* at death, 62 individuals were ground and streaked on gentian violet-agar plates. Of these, 44 (71%) were infected with viable organisms.

The extrinsic incubation period is least well defined for the fleas in experiment A, which were given opportunities to feed on a second mouse only after the death of the first. A maximal extrinsic incubation period may be calculated and as used here is the time from the day the flea took blood containing *Past. pestis* until its last opportunity to feed on the mouse to which the infection was transmitted. The period calculated in this way is frequently several days longer than the actual extrinsic incubation period, but from the data presented in table 5, it is apparent that over 50% of the fleas transmitted the infection within the first two weeks and over 90% transmitted plague within three weeks of their infectious meals.

DISCUSSION

Considering both groups of fleas together, 285 fed on plague-infected mice; 173 (61%) transmitted the infection to

TABLE 5.—*Maximal extrinsic incubation period.** X. cheopis.

Time, weeks	Transmitters	
	Cumulative number	Percent
0–1	16	9
0–2	101	58
0–3	160	92
0–4	171	99
0–5	173	100

* The maximal extrinsic incubation period as used here is the time between the infectious meal of the flea and the last opportunity to feed on the first mouse to which this flea transmitted the infection.

243 mice. The average number of transmissions per flea was 0.85. The plague transmission frequencies of individual *X. cheopis* are: 119 (68%) transmitted once, 41 (24%) transmitted twice, ten (6%) transmitted three times, and three (2%) transmitted to four mice each. In the present plague transmission experiments *X. cheopis* was again an extremely efficient plague vector.

Since fleas harbor *Past. pestis* for varying lengths of time, conservation of this organism in the flea is the most commonly advanced explanation for the survival of infection through interepizootic periods. Fleas have been found infected several months after an early summer epizootic in Russia that had eliminated susliks in an area studied by Tumansky and Poliak (1931). Suslik nests were excavated and the fleas removed in the fall and winter. In November, five months after the epizootic, four fleas were carrying the plague bacillus, but their digestive tracts were unobstructed. At various intervals until February of the following year, 1797 fleas were collected, but no more plague bacilli were found. On the basis of experiments made in the laboratory, Eskey and Haas (1940) stated that most fleas after infection with plague bacilli remained infected until death and that only from 4 to 19% of certain species eventually become free of the organisms. *X. cheopis* was less likely to become plague-free than any other tested flea. This clearing of plague from *D. montanus* and *X. cheopis* was further investigated by Douglas and Wheeler (1943) in a careful study. After both 24 and 48 hours, 60% of the *D. montanus* were free of the organisms, while all *X. cheopis* remained infected.

In the course of the present transmission experiments, quite a few dead fleas and those killed at the termination of a particular experiment were cultured bacteriologically for *Past. pestis*. The results confirmed the observations of other workers—that some fleas become free of the organisms and that others remain infected until death, even though the fleas did not die until several weeks after they became infected. Earlier workers considered that only those fleas that passed feces containing viable plague organisms were actually infected (Eskey and Haas, 1940). However, in later experimental studies (Douglas and Wheeler, 1943) plague-infected *D. montanus* passed feces containing viable plague organisms only about 25% of the time, and only 56% of the infected *X. cheopis* excreted viable organisms. In another study similar observations were made (Burroughs, 1947). Thus, the excreta of infected fleas do not always contain viable plague organisms, and the bacteriological culturing of the triturated flea offers the only reliable method of ascertaining whether a flea is infected with *Past. pestis*.

An effort was made to test the relation of time to the disappearance of the organisms from the fleas. The 160 *D. montanus* and 150 *X. cheopis* used were infected with *Past. pestis* and then held in quart jars (one for each species) containing enough wood shavings to cover the bottom. These jars were placed in a five-gallon crock in a room with a temperature of from 68 to 73 F and a relative humidity of from 60 to 90%. Fleas were removed from the jars and cultured bacteriologically at

intervals. Those to be cultured were cooled in the freezing compartment of a refrigerator for a couple of minutes until immobilized and then were ground. Each triturated flea, diluted with two or three drops of normal saline solution, was smeared on a section of a petri dish containing gentian-violet agar medium. The feeding schedule of these fleas and results of the bacteriologic cultures are given in table 6. After their infectious meals the fleas were given an opportunity to feed collectively

TABLE 6.—*Persistence of* Past. pestis *in* D. montanus *and* X. cheopis.*

Days after infective meals	Fleas cultured for *Past. pestis*			
	Total	Negative	Positive	Percent positive
	D. montanus			
3	15	4	11	73
7	15	6	9	60
12	10	7	3	30
16	10	5	5	50
22	10	7	3	30
25	8	5	3	37
28	10	5	5	50
32	4	3	1	25
35	5	4	1	20
36–43	5	5	0	0
Total	92	51	41	45
	X. cheopis			
3	15	1	14	93
7	15	0	15	100
12	10	0	10	100
16	10	0	10	100
22	10	1	9	90
28	8	0	8	100
32	3	0	3	100
35	1	1	0	0
36	1	0	1	100
49	1	1	0	0
52	2	1	1	50
Total	76	5	71	93

* After the infective meal, feeding opportunities were given the fleas on the following days: 3, 5, 7, 11, 16, 22, 25, 28, 32, 35, 38, 41, 46 and 49.

on muzzled plague-free mice. Most of the fleas did not feed when free on the muzzled mouse on the first two feeding opportunities, but the number feeding was increased on the remaining opportunities when groups of ten fleas were put in a vial and inverted on the shaved belly of a mouse. After feeding, the fleas were returned to the quart jar. By the 25th day after the infectious blood meals, few fleas remained alive. The remaining samples tested were composed largely of fleas that had been dead for less than 24 hours because they were removed daily from the holding jars.

The results of this experiment show that even as early as the third day after an infectious meal, more than a fourth of the *D. montanus* had become free of *Past. pestis*. The last *D. montanus* retaining viable *Past. pestis* died on the 35th day. In contrast, *X. cheopis* in this experiment remained infected until the 52nd day and only five of the total of 76 fleas were plague-free.

By comparison, in the plague transmission experiments, 45 (68%) of the 66 *X. cheopis* contained *Past. pestis* when they were killed from 22 to 31 days after they had ingested infected blood. Only eight of the 60 (13%) of the *D. montanus* used in the plague transmission experiments harbored plague organisms when killed from 22 to 31 days after their infectious meals. A larger portion of the fleas of both species in the special study remained plague-infected than did the fleas in the plague transmission experiments, possibly because the former were not offered blood meals as often. How the spacing of these meals could influence the retention of *Past. pestis* is not known. In the plague transmission experiments, as well as in the special study on the survival of plague organisms in fleas, *Past. pestis* was eliminated from *D. montanus* more rapidly than from *X. cheopis*. This is certainly one factor contributing to the comparatively low rate of plague transmission by *D. montanus*.

In plague transmission experiments with 88 *P. gwyni*, only 18 (20%) were negative when cultured bacteriologically at the time of their death or when killed at the end of the experiments. The disappearance of the plague organisms from the fleas probably depends on factors other than phagocytosis because there is no reason to doubt that this would proceed at about the same rate in all flea species. Neither does mechanical flushing out of the alimen-

tary tract with blood ingested after infection offer an explanation because *P. gwyni* defecates profusely after the first few feedings, yet 80% of these fleas which took blood containing *Past. pestis* remained infected until death. The mechanism by which fleas are cleared of *Past. pestis* is not known, but the two commonly advanced hypotheses do not offer an acceptable explanation.

CONCLUSIONS

Diamanus montanus from six separate geographic areas in central California were tested for plague-transmission ability. Fleas from four of these areas, three with a known history of sylvatic plague and one supposedly plague-free, did not transmit plague. From two other areas, both supposedly free of the disease, 4% of one and 5% of the other transmitted plague. Apparently the plague transmission potential of the different strains of *D. montanus* used in the experiments differs. However, because of the low percentage of fleas transmitting, the significance of the differences cannot be accurately determined. The plague transmission potential of this species is apparently very low.

After plague-infective meals, a greater portion of *D. montanus* became cleared of plague organisms than did *Xenopsylla cheopis*, and the rate of clearing is more rapid in *D. montanus*.

Polygenis gwyni has one of the highest known plague vector efficiencies of the native North American rodent fleas. Twenty-seven percent of the infected fleas transmitted plague. This species has not been studied in a sylvatic plague epizootic and its vector ability under these conditions is not evident, but the data compiled indicate that it would be high.

X. cheopis was the most efficient vector of the three species of fleas tested. Sixty percent of the infected fleas transmitted plague one or more times.

The greatest opportunity for sylvatic plague to spread through the rodent-flea chain into urban centers would appear to be in certain areas in southern and southwestern United States, where both highly efficient vectors *P. gwyni* and *X. cheopis* occur on field-dwelling domestic rats. Though plague is not now known to exist in any of these areas, it is a potential danger that should not be ignored, especially in the southwest adjacent to known plague foci.

The results obtained in the plague transmission experiments by Wheeler and Douglas (1945) and by Burroughs (1947) and in those reported herein demonstrate that many unknown variables influence the plague vector efficiency of a single species of flea. Comparisons must be made with caution.

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TRANSMISSION AND CONTROL OF RESPIRATORY DISEASE IN ARMY BARRACKS

III. THE SUPPRESSION OF DUST-BORNE BACTERIA BY OILING FLOORS AND BEDCLOTHES

HENRY M. LEMON,* CLAYTON G. LOOSLI, HENRY WISE† AND THEODORE T. PUCK‡

From the Commission on Air-Borne Infections, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, and the Department of Medicine, University of Chicago, Chicago 37, Illinois

During the winter of 1943–44 studies concerning the epidemiology and control of respiratory tract infections were made at Camp Carson, Colorado, by the Commission on Air-Borne Infections.[1] Reports on the streptococcal contamination of the barracks' environment and the possible modes of spread of streptococcal infections among the enlisted personnel have been published.[2,3] During these investigations it was shown that the environment of the barracks, including the air, floor dust, blankets, and wearing apparel, became markedly contaminated with streptococci. The data suggested strongly that these environmental reservoirs of bacteria were an important source of organisms causing streptococcal infections among the occupants of the barracks.

At the time of this study it had already been demonstrated in England by Van den Ende et al[4] and Thomas and Van den Ende[5] that the application of oil to floors and bedding was highly effective in controlling the dispersal of dust-borne bacteria from treated floors and bedding. The practical details of applying oil to floors and bedding (blankets and comforters) on a large scale in a military establishment and the bacteriological evidence for the effectiveness of these oiling procedures in diminishing the opportunities for the transfer of air-borne pathogens over long periods of time are the subjects of this report.

MATERIALS AND METHODS

As the general plan of study included an evalu-

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* Present address: Evans Memorial Hospital, 65 E. Newton St., Boston, Mass.

† Present address: Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Calif.

‡ Present address: Dept. of Biophysics, University of Colorado, Denver, Colo.

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ation of dust-suppressive measures in controlling respiratory tract infections, it was necessary to oil the floors of barracks billeting approximately 9000 men in as short a time as possible. During the study over 293 buildings (barracks, day rooms and mess halls) were oiled. The personnel employed for the treatment of floors and bedclothes were made available to the Commission from the 745th Sanitary Company and were under the command of Captain J. E. Davis. The barracks, of wood construction two stories in height, had a regulation capacity for 60 men and had unpainted floors of soft pine. The materials and methods used for applying oil to floors and bedclothes will be described in sufficient detail to allow wide application in military establishments.

Oiling floors

Kind of oil.—A pale paraffin oil* which met the United States Government standards for floor oil was employed.[6] Its specifications were as follows:

Specific gravity	27–29
Flash point (open cup)	350–360° F
Fire point	405–415° F
Pour point	−5° F
Viscosity at 100° F	100–110
Viscosity at 210° F	38
Color	pale yellow

The oil was free from sediment, foreign material and objectionable odor. It was purchased and shipped in 55-gallon steel drums.

Equipment.—The materials for cleaning and oiling a barracks are shown in figure 1. An oiling crew consisting of six men required the following equipment: two buckets, 15-quart capacity, two no. 10 size fruit cans with holes punched in the bottom, four 16-inch hair brooms. If the barracks were to be cleaned before oiling, two mop buckets with wringers, four cloth mops, four corn brooms and one half pound of G.I. soap per barracks were supplied for each crew. A truck with driver was necessary to carry equipment and personnel to and from the area to be oiled (fig. 1).

Procedure for oiling floors.—As the barracks were already occupied, it was necessary to make arrangements with the regimental and respective company commanders several days in advance so that the barracks could be properly "stripped" and cleaned in preparation for oiling. The day before the barracks were to be oiled, the floors were scrubbed. Everything except equipment which hung on the walls was removed from the building. The floors were then swept, scrubbed thoroughly with corn brooms, using ample soap, and allowed to dry while the occupants were in the field.

On the day of oiling the barracks were again "stripped." Oil was taken to the area in a truck and a dispensing station was set up to serve two to four oiling crews. The respective duties of the members of a crew were as follows: one man carried oil from the station in 15-quart buckets; a second man spread it over the floor from perforated gallon cans at right angles to the floor boards (fig. 2); two men then followed closely and "brushed" the oil over the floor with 16-inch hair brooms; a second more thorough brushing was done by the remaining two men.

With a crew of six and the above equipment it required from 30 to 50 minutes to oil both floors of a barracks having a surface of approximately 5310 square feet. The amount of oil employed averaged approximately 30 gallons per barracks or one gallon per 180 square feet of floor space. After five to six hours the floors were sufficiently dry to allow the barracks to be occupied without undue soiling of clothes and equipment. After the oiling, the barracks occupants were instructed to clean the floors only by sweeping daily with a dry or oiled broom and mopping them once a week with frequent changes of warm water without soap to avoid removal of the oil.

Oiling of bedding

Only blankets and comforters were treated with oil. The oil emulsion base† for preparing the dilute oil-in-water emulsion had the following formula:

Mineral oil (Fractol A)	88.0 g
Oleic acid (purified)	8.9 g
Triethanolamine	3.9 g
Lecithin	0.01 g

The oil was applied as a 1% oil-in-water emulsion in the final rinse water following the routine washing procedure. Figure 3 shows members of the laundry crew measuring out the white, creamy emulsion base which is to be added to the final rinse water in the laundry wheels. When special care was taken the above oil emulsion base was satisfactory, for after the blankets were dried they were found by extraction to contain from 2 to 4% oil by weight. For use as a routine

* Obtained from the Continental Oil Co. Inquiry revealed that most oil companies manufactured an oil especially for floors.

6. Federal Specifications for Oil, Floor, Mineral. 1938, Federal Standard Stock Catalog, Section IV (part 5, P-O-361 July 5).

† Prepared for the Commission and shipped in 55 gallon drums by R. R. Street and Company, Chicago, Illinois.

FIG. 1.—Equipment and crew for cleaning and oiling barracks floors.

FIG. 2.—Procedure for application of the oil to the floors.

laundry procedure, however, triethanolamine oil base had distinct disadvantages in that it tended to separate in the drums and on storage the treated blankets developed a rancid odor due to the presence of oleic acid.

For these and other reasons it was discarded eventually in favor of the Triton oil emulsion base (T-13)* employed in a subsequent study at Fort Bragg, North Carolina.[7] The detailed procedure for using the triethanolamine oil base, therefore, will not be given. Studies of the de-

* Now made by Baxeal Laboratories, Chicago, Ill.

7. The Commission on Acute Respiratory Diseases and the Commission on Air-Borne Infections. 1946, A study of the effect of oiled floors and bedding on the incidence of respiratory disease in new recruits. Am. J. Hyg. **43**: 120–144.

FIG. 3.—Members of the laundry crew measuring out the oil emulsion base which will be added to the final rinse water.

FIG. 4.—Some of the morning activities in the barracks at the time air samples were taken. The Folin bubbler sampler and motor are seen in the foreground.

velopment of the T-13 oil emulsion base for making dilute oil-in-water emulsions and the directions for the routine application of oil to cotton and woolen textiles have been published [8,9] and should be referred to for details concerning practical methods for applying oil to blankets, sheets, etc.

Because the laundry facilities on the post were completely occupied during the day and evening it was necessary to organize a "graveyard shift" from 11 P.M. to 7 A.M. for treatment of the blankets with oil. The personnel of the 745th Sanitary Company served as the laundry crew with one of the Commission members in charge. During this study approximately 26,000 blankets and comforters were treated with oil. About 1500 blankets and 750 comforters were treated during one shift. The following day "day crews" from the Sanitary Company would distribute the treated bedding to the barracks and exchange it for dirty blankets which were stored in the laundry for the evening's oiling.

Methods for sampling and culturing air and dust from bedding and floors

The methods used for sampling air and collecting dust from bedding for bacteriological analyses have been described in detail in the first paper[2] of this series. The Folin bubbler sampler employed in studies by the Commission on Air-Borne Infections[10–12] was used both for sampling air and collecting dust. The principle of this apparatus is the trapping of bacterial particles in broth as air is drawn through it at a given rate of speed, usually one cubic foot per minute. Usually 10 to 20 cubic feet of air were drawn through the bubbler for sampling. For sampling blanket and floor dust a small funnel was attached to the inlet by a rubber hose. The funnel was then slid gently over the surface of the blanket and loose lint, bacteria, and other particulate matter were drawn into the broth. A sampling period of 30 seconds was adequate for blankets and floors and one minute for sheets and pillow cases. The latter were not present in most barracks.

The sampler broth was then plated in nutrient agar to which 5 ml of defibrinated mule blood was added. In the case of air cultures, aliquots of 0.5 ml were used for the determination of total numbers of microorganisms and 2 ml seedings for the detection of β-hemolytic streptococci.* To facilitate the isolation of hemolytic streptococci, 1:500,000 dilution of Gentian violet was added to the blood agar, which acted to suppress the growth of other bacteria.[13] Similar procedures were employed for cultures of dust except that smaller amounts of the sampler broth were used—0.05 ml for total bacteria and 0.2 ml for streptococci. Usually one streptococcus colony was picked from "positive" plates for grouping and typing by the method of Swift et al.[14]

Sampling of the air of barracks for the most part took place over a two to 2½-hour period from 5 to 7:30 a.m. when the men were sleeping, getting up, dressing, sweeping floors, making beds, out to meals and leaving the barracks for the day's activities. Figure 4 is a photograph showing the beginning morning activity in the barracks. The typical location of an air sampler and pump is shown in the foreground, in the center aisle near the return air grill of the warm air heating system. During the winter months when the temperature was low the windows and doors were usually closed providing little supplementary ventilation when the samples were

8. Puck, T. T. Robertson, O. H., Wise, H.. Loosli, C. G. and Lemon, H. M. 1946, The oil treatment of bedclothes for the control of dust-borne infection. I. Principles underlying the development and use of a satisfactory oil-in-water emulsion. Am. J. Hyg. **43**: 91–104.
9. Loosli, C. G., Wise, H., Lemon, H. M., Puck, T. T. and Robertson, O. H. 1946, The oil treatment of bedclothes for the control of dust-borne infection. II. The use of Triton oil emulsion (T-13) as a routine laundry procedure. Am. J. Hyg. **43**: 105–119.
10. Lemon, H. M. 1943, A method for collection of bacteria from air and textiles. Proc. Soc. Exper. Biol. & Med. **54**: 298–301.
11. Lemon, H. M. and Wise, H. 1944, A flow meter for use in air sampling procedures. Science, **99**: 43–44.
12. Lemon, H. M., Wise, H. and Hamburger, M. 1944, Bacterial content of air in army barracks. Results of a study with especial reference to the dispersion of bacteria by the air circulation system. War Med. **6**: 91–101.

* The terms streptococcus and hemolytic streptococci indicate β-hemolytic streptococci.

13. Garrod, L. P. 1933, Inhibitory selective culture media. I. A review of methods proposed for bacterial cultivation which utilize differential inhibition. II. Original observations: the selective cultivation of streptococcus pyogenes. St. Bartholomew's Hosp. Reps. **64**: 203–252.
14. Swift, H. F., Wilson, A. T. and Lancefield, R. C. 1943, Typing Group A hemolytic streptococci by M precipitin reactions in capillary pipettes. J. Exper. Med. **78**: 127–133.

taken. From April through August the temperature was such that many of the windows were open at night. During this study both oiled and unoiled barracks were sampled at similar periods and corresponding comparisons of counts made so that changes in numbers of bacteria due to by the feel or general appearance to detect the presence of oil in the treated blankets and comforters. Certain lots of treated blankets which had been stored several days before use developed a

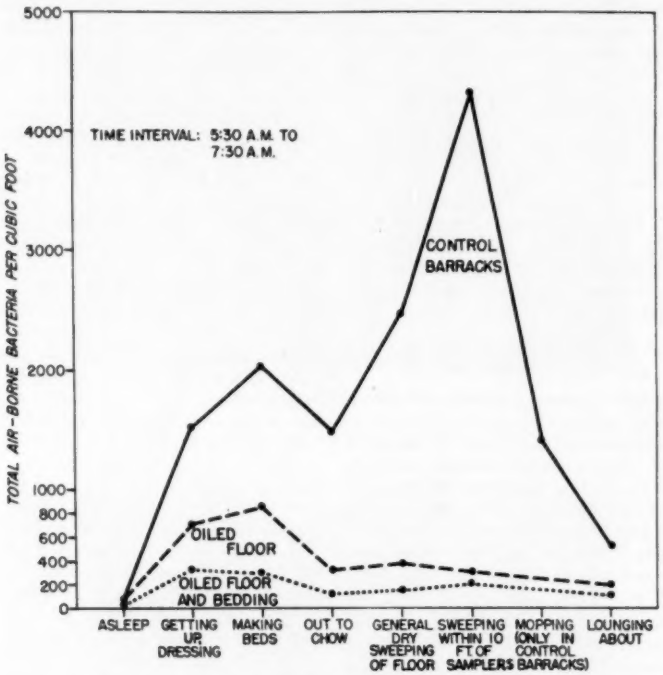

FIG. 5.—The mean bacterial counts in the air of unoiled and oiled floor barracks with oiled bedding at different periods of activity from 5:30 to 7:30 a.m.

supplementary ventilation in the test and control barracks would be the same.

The dust and bacteria holding capacity of oiled floors and blankets in barracks was studied over a period of approximately eight months. During this time several thousand cultures of air, floor dust and bedding from several dozen pairs of oiled and unoiled barracks were made. Blankets and floor dust were sampled usually after the men had left the barracks.

RESULTS

Reaction of barrack personnel to oiled floors and bedding.—It was impossible slightly rancid odor which largely disappeared when they were "aired" for several hours or after a day on the bunks. There were some complaints for a few days following oiling of the floors because of soiling of uniforms and other clothing which was allowed to come in contact with floors. In general, however, the reaction of the barracks occupants to the oiled floors and bedding was favorable. They commented particularly on the dust free atmosphere and

the ease of maintaining cleanliness in the barracks.

Reduction of dust-borne bacteria by a single application of oil to floors.—As seen in figure 5, when the personnel were all asleep and there were no dust raising activities of any kind the bacterial content of the air was essentially the same in the oiled and unoiled barracks. With increasing activity there was a marked rise in the bacterial content of the air in the unoiled barracks. After the activities in the barracks had ceased the bacterial content returned to a low level in a short time. Thus, as shown in table 1, on the basis of several hundred air

TABLE 1.—*Mean bacterial content of air of oiled- and unoiled-floor barracks during maximum periods of activity taken over an eight month period.*

Barracks	Number of different barracks	Number of tests*	Mean total bacteria per cu. ft.	Percent difference
Unoiled	22	66	1792	
Oiled	21	30	498	72.2

* A "test" run consisted of taking from six to eight 20-minute air samples (20 cubic feet per sample) consecutively in a squad room between the hours of 5:00 and 7:30 a.m.

samples taken over the two-hour morning period, there were approximately 75% less bacteria in the air of the oiled floor barracks as compared to the number isolated from the air of unoiled barracks. A reduction of over 90% was accomplished at times of maximum dust raising activity by the suppressive action of oiled floors. Even during the early activities when dressing and bed making were included there was more than a 50% reduction in air-borne bacteria in the oiled barracks than in the unoiled ones (fig. 5).

Over an eight-month period of observation one application of oil to the floors remained effective in reducing the mean bacterial content of the barracks air during the morning hours of activity

TABLE 2.—*Duration of effectiveness of oiled floor in suppressing aerial contamination of barracks air.*

Barracks	Number of different barracks	Number of tests*	Mean total bacteria per cu. ft.	Percent difference
Unoiled	22	66	1792	
Oiled Duration				
0–1 month	7	11	396	82.0
1–2 month	4	4	699	61.0
2–3 month	2	4	467	74.0
3–4 month	4	7	418	77.7
8 month	4	4	512	71.5

* See footnote, table 1.

by approximately 70%, compared to air counts of the control barracks (table 2). This was essentially the same percent reduction as was noted one month after application of oil to the floors. However, the need for re-oiling parts of the floors became apparent after six months in some barracks where the floors had begun to deteriorate before the oil was applied. The need for re-oiling was also apparent sooner in locations where maximum wear occurred, such as stairways, center aisles and the main entrance corridor through which soapy water was swept daily from the undrained washroom. The dust-holding efficiency of oiled floors generally could be observed by the degree to which the dust during sweeping was rolled into cohesive lumps or balls. In such barracks air cultures always yielded low counts. Thus, in the absence of bacteriologic criteria, this means could be employed to judge the continuing effectiveness of oiled floors.

Reduction of dust-borne bacteria by oiling floors and bedding.—The addition of oiled blankets and comforters to the beds in oiled floor barracks resulted in a further decrease in the number of bacteria dispersed into the air during the morning activities (fig. 5, table 3). This reduction was most marked during the waking, dressing and bed making periods when agitation of the blankets was at a maximum but persisted to a

TABLE 3.—*Reduction of dust-borne bacteria by oiled floors, and oiled floors plus oiled bedding one to three months after application.*

Barracks	Number of different barracks	Number of tests*	Mean total bacteria per cu. ft.	Percent difference
Unoiled floors Unwashed bedding	4	23	1777	
Oiled floors Oiled bedding	5	16	245	87.2
Unoiled floors Newly washed bedding	2	5	2600	
Oiled floors Newly washed bedding	2	7	302	88.4
Oiled floors Oiled bedding	6	14	226	91.3

* See footnote, table 1.

lesser extent for the entire period. As noted in figure 5, the mean bacterial count in the barracks with oiled floors and bedding was reduced only slightly beyond that attained by floor oiling alone. This indicates that the greatest source of air contamination came from dispersion during floor sweeping, which was controlled by oiling the floors.

In spite of oiled floors and bedding, there was a tenfold increase in the bacterial content of the air in the oiled barracks during the early part of the morning activity (fig. 5). The source of this contamination was in all probability from clothing, mattress covers, pillow slips (sheets were not provided) and other unoiled sources of bacterial contamination. In addition to the marked reduction in the mean numbers of bacteria from the air during the two-hour sampling period, there was a similar reduction in the maximum number of organisms isolated from the air. In the unoiled barracks, air counts as high as 5000 to 10,000 bacteria per cubic foot were not unusual during periods of maximum dust-raising, such as when sweeping occurred immediately beneath the sampler, whereas under similar conditions of sweeping in the oiled barracks, 500 to 800 bacteria per cubic foot of air was the upper limit and values as high as these were encountered only in the first part of the sampling period when agitation of untreated reservoirs such as clothing was most pronounced.

Effect of oiled floors and bedding on the streptococcal content of the air.—Air cultures were taken with the Folin bubbler sampler simultaneously in oiled and unoiled barracks of a single battalion during a period of two months. As seen in table 4 over 40% of the air samples from the unoiled barracks were positive for group A streptococci, whereas only 9% of those from the oiled barracks were positive. Of the positive cultures over nine times as many streptococci were present in the samples obtained from the unoiled barracks as were isolated from the air of oiled barracks. The total bacterial count from the air of the untreated barracks also was proportionately higher than the total counts in the oiled environments.

The few streptococci recovered from the air of the oiled barracks were obtained, like those found in the unoiled barracks, from the samples taken during periods of maximum dust raising activity, such as dressing, bed making and sweeping. Thus, these organisms may be considered truly dust-borne and not suspended in the air as "droplet nuclei." The "settling out" from the air of all

TABLE 4.—*Comparison of the total mean number of bacteria and streptococci isolated from the air of barracks with oiled floors and bedding, and unoiled floors and bedding one to three months after application.*

Barracks	Number of cultures	Cultures positive for streptococci		Mean number per cubic foot	
		Number	Percent	Streptococci	Total bacteria
Unoiled	124	51	41.0	8.0	2190
Oiled	76	7	9.2	0.6	229
Percent difference			77.7	92.5	89.5

the bacteria when dust-raising activities had ceased indicates the importance of "dust reservoirs" as the source for the dissemination of pathogens throughout the barracks environment during the morning activities.

Total bacteria obtained from oiled and unoiled bedding.—Further evidence for the effectiveness of oil-treated blankets in holding bacteria is shown in the marked reduction in numbers obtained on direct culture. During this study several hundred cultures from oiled and unoiled blankets were made employing the Folin bubbler sampler. Cultures of new blankets before they were issued to the men revealed only a small number of organisms. Once in use the bacterial content of untreated blankets rose rapidly (table 5).* A comparison of the bacterial content of the blanket dust revealed a striking difference in numbers from the oiled blankets even after four months of use. Treated blankets containing various amounts of oil were sampled along with freshly laundered bedding after one week's exposure on beds of a hospital ward for respiratory disease (table 5). Over 90% fewer organisms were recovered from the oiled blankets during this period. Likewise, oiled blankets placed in barrackes with oiled floors for four weeks yielded over 85% fewer bacteria on culture than did newly washed blankets which were on bunks in unoiled barracks for a similar length of time (table 5).

There was also a marked reduction in the amount of dirt liberated from the oiled blankets as judged by the reduction in the amount of discoloration of the bubbler fluid in which the dirt was trapped. Correlated with this was a demonstrable retention of dust by the oiled blankets when the beds were struck a sharp blow with a stick or saber. Instead of a choking cloud of dust rising, as it did as a rule from the unoiled blankets, only a few coarse particles and sand could thus be beaten from the meshes of the oiled fabric. These particles were large and immediately settled out of the air. This test was found to offer a quick and reliable method of estimating the amount of oiled bedding remaining in the barracks several weeks to months following its issue.

TABLE 5.—*Recovery of bacteria from oiled and unoiled blankets.*

Blanket source	Kind of treatment	Duration of use	Number of blankets cultured	Mean total bacteria recovered from blankets	Percent difference
New unused	None	0	19	5,920	
Hospital ward	Freshly washed	1 wk	9	1,088,000	
Floor varnished	Treated 1 to 20% oil	1 wk	20	92,000	84
Barracks					
A-Unoiled floor	Freshly washed	4 wk	12	2,540,000	
B-Oiled floor	Oiled 1%	4 wk	12	522,000	87
C-Unoiled floor	Washed	4 wk	11	1,740,000	
D-Oiled floor	Oiled 2%	4 wk	12	265,000	86

Recovery of streptococci from oiled and unoiled bedding.—In addition to culturing for total bacteria, aliquots of the broth were plated in Gentian violet medium for β-hemolytic streptococci. The analysis of cultures for streptococci from several hundred beds is shown in table 6. The cultures in the control and treated barracks were taken over the

* With the method of sampling, the number of bacteria recovered from the blankets and shown in table 5 represents only a small fraction of the total recoverable bacteria, as can be shown by the repeated sampling of the same blanket.

TABLE 6.—*Effect of oil treatment on the number of β-hemolytic streptococci cultured from blankets.*

Blankets	Number cultured	Cultures positive for streptococci		Mean number of streptococci in positive cultures
		Number	Percent	
Unoiled	223	170	76	4520
Oiled	224	45	20	250
Percent difference			74	95

same period of time from May 24 to June 6, 1944. Table 6 shows the number of streptococci collected from oiled and unoiled blankets approximately six weeks after being placed in barracks of the same battalion with and without oiled floors. There were 73% less positive bed cultures for streptococci for the oiled barracks and approximately one-fifth as many streptococci were recovered from the oiled beds as were cultured from the unoiled blankets. Seldom was a culture from an oiled blanket found to contain large numbers of streptococci as was frequently observed in cultures from untreated bedding.

It should be pointed out that these data may not reflect the true bacteria-holding properties of the blankets. First, the streptococcal carrier rate was lower among the occupants of oiled barracks and, in addition, the oiled floors undoubtedly contributed to a reduction in streptococcal pollution of the bedding. However, every barracks with completely oiled bedding and floors showed at all times significantly fewer numbers of "positive beds" compared to the findings in the untreated barracks. When positive beds were detected in the test barracks, the streptococcal content was, as a rule, low.

DISCUSSION

At Camp Carson a single application of a liberal amount of floor oil to the barracks' floors was highly effective in preventing the dispersion of dust and dust-borne bacteria during sweeping periods for as long as eight months. After this time the oil had worn thin in some areas where traffic was heavy (center aisles and near the latrine entrance). If these areas were touched up with another coat of oil, it was considered that the oiled floors would continue to hold dust and lint for another several months.

The addition of oil to the bedding further reduced the bacterial content of the oiled barracks' air during the morning bed making. At Camp Carson the dust-holding capacity of oiled blankets was observed to be effective over a period of four months' use. Likewise, during the investigation at Fort Bragg, North Carolina, of the usefulness of the Triton (T-13) emulsion base formula for treatment of bedclothes with oil, it was found that blankets containing from 2 to 3% oil by weight retained their ability to hold dust and lint for as long as 18 weeks after a single treatment.[7,9] In barracks with oiled floors they showed no gross soiling at the end of this time. Further studies at Fort Bragg were made on the oil content of treated blankets after washing and on the bacteria-holding efficiency of oiled blankets which had been washed and placed in the barracks without retreatment. It was found that oil content of treated blankets was not removed during the routine washing procedure, and at the end of washing they appeared as clean as the untreated ones. Such rewashed oiled blankets were found to be just as effective in holding bacteria and dust as were freshly oiled ones.[7] However, it was noted both at Camp Carson and Fort Bragg that oiled blankets, when placed in the dusty atmosphere of unoiled floor barracks, showed a diminished bacteria-holding efficiency and were grossly dirty after four to six weeks use. Thus, the length of time oil-treated blankets remain effective in holding dust will de-

pend on whether dust suppressive measures are also applied to the floors. These findings would indicate that in order for oiled blankets to maintain their dust-holding properties throughout a respiratory disease season they should be used in conjunction with dust-holding measures applied to floors.

In the two previous reports[2,3] of this study it was shown that the environment of the barracks, including bedding, floor and clothes, became highly contaminated with streptococci at the time when streptococcal disease was occurring among the troops. With streptococcal typing procedures and from a thorough study of the bacterial content of the barracks' environment, it was concluded that the majority of the streptococcal infections were acquired in the barracks. It is recognized that in such intramural environments there are many possibilities for the spread of infection among the occupants. The practical value, therefore, of dust-suppressive measures for the control of streptococcal infections in the barracks would depend on how many are acquired directly or indirectly through dust and air. The demonstration of the very high total bacterial and streptococcal (type-specific) content of the air in the unoiled barracks during periods of dressing, floor sweeping and bed making, the general dissemination of a single type of streptococcus throughout the barracks, and the occurrence of respiratory infections due to this type constitute evidence that the mode of transfer via dust and air is important. A detailed account of the spread of streptococcal infections among the personnel in highly contaminated barracks at Camp Carson has been made.[3] Hamburger and associates[15,16] have shown that dust and air of wards housing patients with streptococcal disease also become highly contaminated with streptococci and have demonstrated the importance of "nasal carriers" of streptococci in the contamination of the ward environment. It is of interest to note that the mean streptococcal content of positive air cultures in the untreated barracks (table 4) considerably exceeds similar estimates made of aerial streptococcal contamination in scarlet fever wards by Hamburger and associates, who used identical equipment. The difference can best be explained by the far greater reservoirs for streptococci in the barracks.

Studies by various investigators concerning the problem of dust control for the disinfection of air and thus the prevention of respiratory tract infections in barracks and hospitals have been reviewed.[17–19] The combined use of oiling procedures and chemical disinfection of the air for the control of air-borne streptococci in hospital wards was made by Puck and associates.[20] They found

15. Hamburger, M., Jr. Puck, T. T., Hamburger, V. G. and Johnson, M. A. 1944, Studies on the transmission of hemolytic streptococcus infections. III. Hemolytic streptococci in the air, floor dust and bedclothing of hospital wards and their relation to cross infection. J. Infect. Dis. **75:** 79–94.
16. Hamburger, M., Jr., Green, M. J. and Hamburger, V. G. 1945, The problem of the "dangerous carrier" of hemolytic streptococci. II. Spread of infection by individuals with strongly positive nose cultures who expelled large numbers of hemolytic streptococci. J. Infect. Dis. **77:** 96–108.
17. Loosli, C. G. and Robertson, O. H. 1945, Recent studies on the control of dust-borne bacteria by treatment of floors and bedclothes with oil. Am. J. Med. Sc. **209:** 166–172.
18. Loosli, C. G. 1947, Dust and its control as a means of disinfection of air. Am. J. Pub. Health, **37:** 353–359.
19. Loosli, C. G., 1948, Problem of dust control for the disinfection of air. Am. J. Pub. Health, **38:** 409–415.
20. Puck, T. T., Hamburger, M., Jr., Robertson, O. H. and Hurst, V. 1945, The effect of triethylene glycol vapor on air-borne beta

that dust-suppressive measures alone lowered the streptococcal count by 86% during bed making, and in combination with glycol vapors, 93%. Less marked reductions in the bacterial content of the air of Naval barracks were reported by Miller,[21] Shechmeister[22] and associates when dust-suppressive measures were employed alone or in combination with ultraviolet light.

The results of the use of oiling procedures applied to barrack floors and bedding at Camp Carson as a means of reducing the morbidity from the common respiratory tract infections among the occupants will be given in the report that follows.[23]

SUMMARY AND CONCLUSIONS

In 1943–44 a study was made of the use of oil to floors and bedclothes in barracks for the suppression of dust and dust-borne bacteria.

It was shown that a single application of oil to floors (one gallon per 180 square feet) was highly effective in controlling the aerial dispersion of bacteria during periods of floor sweeping for as long as eight months.

Oiled blankets (containing from 2 to 4% oil by weight) were also effective in holding bacteria during bed making for as long as four months. When employed in barracks with oiled floors, oil-treated blankets should retain their dust-holding properties and apparent cleanliness for the whole period of a respiratory disease season.

The methods for the practical application of oil to floors and bedding have been described. The simplicity, durability and the effectiveness of these dust-suppressive procedures are emphasized.

hemolytic streptococci in hospital wards. II. The combined action of glycol vapors and dust control measures. J. Infect. Dis. **76**: 216–225.

21. Miller, W. R., Jarrett, E. T., Willmon, T. L., Hollaender, A., Brown, E. W., Lewandowski, T. and Stone, R. S. 1948, Evaluation of ultraviolet radiation and dust control measures in control of respiratory disease at Naval Training Center. J. Infect. Dis. **82**: 86–100.
22. Shechmeister, I. L. and Greenspan, F. S. 1947, The relation of the oil treatment of floors and bedding to the control of respiratory diseases among Naval personnel. Am. J. Hyg. **46**: 376–407.
23. Loosli, C. G., Lemon, H. M., Robertson, O. H. and Hamburger, M. 1952, Transmission and control of respiratory diseases in Army barracks. IV. The effect of oiling procedures on the incidence of respiratory diseases and hemolytic streptococcal infections. J. Infect. Dis. **90**: 153–164.

TRANSMISSION AND CONTROL OF RESPIRATORY DISEASE IN ARMY BARRACKS

IV. THE EFFECT OF OILING PROCEDURES ON THE INCIDENCE OF RESPIRATORY DISEASES AND HEMOLYTIC STREPTOCOCCAL INFECTIONS

CLAYTON G. LOOSLI, HENRY M. LEMON,* O. H. ROBERTSON, AND MORTON HAMBURGER†
From the Commission on Air-Borne Infections, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, and the Department of Medicine, University of Chicago, Chicago 37, Illinois

During the winter of 1943 and 1944 an extensive study was carried out by the Commission on Air-Borne Infections on epidemiology and control of respiratory tract infections among enlisted personnel.[1] In previous reports[2] it was shown that the barracks' environment (floor dust, beds and air) at this military installation became highly contaminated with β-hemolytic streptococci during the winter months. It was shown also that these environmental reservoirs of streptococci were, in all probability, of importance in the spread of streptococcal disease among the men billeted in the barracks.[3]

In a third paper, data were presented which showed that oiling floors and blankets was a highly effective procedure in keeping the bacterial content of the air in the barracks at a low level during periods of maximal activity, such as when the beds were made and the floors swept.[4] The present report gives the results of a study to evaluate the use of these dust-suppressive measures as a means of controlling respiratory tract infections considered to be air-borne or dust-borne in their mode of spread among men living in oiled and unoiled barracks from January 1, 1944 to July 1, 1944.

MATERIALS AND METHODS

A detailed description of the materials and methods employed in this study for applying oil to floors and bedding and for analysis of the air, floor dust and

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* Present address: Evans Memorial Hospital, 65 E. Newton St., Boston, Mass.

† Present address: Dept. of Internal Medicine, Cincinnati General Hosp., Cincinnati 29, Ohio.

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bedding for bacteria have been given in previous reports.[1–4]

PLAN OF STUDY

Choice of barracks.—It was proposed at the beginning of the study to oil the floors and bedding in every other barrack throughout a whole infantry division of approximately 9000 men. Since the laundry procedure of treating bed clothes with oil was not adequately worked out at that time, the study was begun with oiled floors alone. In addition, the floors of the day rooms and recreation buildings and mess halls were oiled. Shortly after the study got underway the division (71st) under observation left Camp Carson. Because of the constant movement of the remaining troops, the study was extended to Peterson Field at the invitation of the medical authorities of the Second Air Force. Subsequently the barracks with oiled floors were reoccupied by a new division which, in conjunction with the undertaking at Peterson Field, increased considerably the scope of the original study.

Soon after the oiling of every other barrack in the divisional area was completed and observations on the control of infections were begun, it became apparent that a single barrack was not an entirely satisfactory epidemiological unit because of the shifting of men, at times, from one platoon to another within the company. Subsequent areas at Camp Carson and Peterson Field, therefore, were oiled, respectively, according to company* and block, which were the smallest units of supply and command. With the company as the epidemiological unit the changes in personnel could be more adequately followed and the oiled bedding could be more easily confined to a company's barracks. The study of the combined effects of oiled bedding and oiled floors began at Peterson Field February 28 and at Camp Carson April 1, 1944.

Choice of troops.—By the selection of an infantry division on which to make observations, the personnel in the test and control groups could be considered essentially the same with respect to (a) age groups, (b) degree of training, (c) seasoning and (d) kinds of daily activities. Because of the movement of troops in and out of the oiled areas, observations on the incidence of respiratory infections in four different Army and Air Force groups were finally made. They were: (a) 71st Infantry Division and attached artillery units from January 1 to February 5, 1944; (b) Peterson Field Air Force groups from February 28 to June 3, 1944; (c) 104th Infantry Division and attached artillery units from April 1 to June 3, 1944; (d) 286th Combat Engineer Battalion from March 1 to July 1, 1944.

The experiment, as it finally evolved, consisted of an observational group of approximately 24,500 men divided into test and control groups. Sixteen-thousand and four hundred provided data on oiled versus unoiled floors, and approximately 8000 constituted the test of the effect of oiling both floors and bedding. There was some variation in the length of service of the men in the different organizations, but on the basis of age, degree of training, similarity of activity and troop requirements within a given organization, the personnel in the treated and control groups may be considered as comparable. The great majority were "seasoned" men, having been with their companies from 6 to 18 months. One observational group was an engineer battalion composed essentially of new recruits. This organization had been activated at Camp Carson in the early part of March, 1944 with new inductees directly out of civilian life.

Ideally a daily flow chart of the men coming into and leaving an organization should have been made. Because of the limitation of personnel conducting the study, only daily strength records of the companies, and, in certain cases, platoons were used in comparing the strength of the test and control groups. When replacements occurred in the various organizations, they were usually distributed equally within the various companies.

Criteria of effectiveness of oiling procedures.—The criteria of effectiveness of the oiling procedures in controlling infections was based for the most part on hospital admissions for diseases of the respiratory tract, including nasopharyngitis* of streptococcal and nonstreptococcal origin. Other infections having a portal of entry through the respiratory tract and therefore possibly airborne or dust-borne in transmission were also tabulated. In the case of the engineer battalion whose enlisted men consisted essentially of new recruits, visits to the dispensary from the oiled and unoiled barracks were also tabulated. Three monthly streptococcal carrier surveys in this battalion during May, June and July were also employed as a possible means of evaluating the effectiveness of the oiling procedures.

RESULTS

71st Infantry Division and artillery groups, oiled floors alone.—In this in-

* A company usually occupied three barracks.

* The great majority of admissions were diagnosed simply as nasopharyngitis, an Army term for an upper respiratory infection.

fantry division area, oiling of alternate barracks and dayroom floors was begun December 11 and completed December 23, 1943. During this time the floors of 64 barracks and 48 dayrooms were treated with oil. Observations on the effect of the oiling procedures on the incidence of respiratory tract infections began January 1, 1944 and continued through February 5, 1944—a period of 5 weeks. The average weekly number of men living in the oiled (4434) and unoiled (4428) barracks was approximately the same. The number of hospital admissions from the treated and untreated barracks with the admission diagnosis is shown in table 1. A total of 185 cases was admitted from the control area, compared to 157 cases from the test barracks. The average weekly admission rate per thousand population was 8.25 from the control barracks, compared to 7.14 from the test group. These differences are of no significance.*

Peterson Field, 2nd Air Force, oiled floors plus oiled bedding.—After the 71st Infantry and Artillery Division left Camp Carson, it appeared uncertain whether sufficient new troops would be available at Camp Carson for further study. Therefore, a program of respiratory disease control in the barracks at Peterson Field, 2nd Air Force, was planned. Oiling of the barracks began February 16 and was completed February 19, 1944. Treatment of the blankets was begun February 18 and completed February 25. Thus, the floors of 62 barracks (theater of operations type) were oiled and the personnel supplied with treated blankets. The barracks were treated by blocks, which facilitated the handling and distribution of the blankets and the keeping of records. In terms of length of service and activity, the men living in the oiled areas were not necessarily comparable to those living in the unoiled barracks, as will be explained in more detail below.

TABLE 1.—*Weekly hospital admissions for upper respiratory and other infectious diseases from unoiled and oiled barracks in the 71st Division area January 1 to February 5, 1944.*

Admission diagnosis	Unoiled barracks						Oiled-floor barracks					
	Jan. 1–8	Jan. 9–15	Jan. 16–22	Jan. 23–29	Jan. 30–Feb 5	Total	Jan. 1–8	Jan. 9–15	Jan. 16–22	Jan. 23–26	Jan. 30–Feb 5	Total
Nasopharyngitis	36	37	26	36	8	143	20	22	36	37	14	129
Scarlet fever						0						0
Tonsillitis	1	7	5	3		16	1	2	1			4
Bronchitis	3	3		1		7	4	6	2			12
Laryngitis	2	1	1			4	2	1	4			7
Mumps		1	1	6		8	2				1	3
Measles				1		1		1			1	2
Pneumonia		1	1	2	1	5						0
Total	42	50	34	49	9	184	29	32	43	37	16	157
Weekly admission rate per 1000	9.5	11.3	7.7	11.1	2.0	Average 8.4	6.5	7.2	9.7	8.3	3.6	Average 7.1

Avg. weekly strength of unoiled barracks—4428, of oiled—4434.

As shown in table 2, observations on the control of respiratory tract infections were begun February 28 and extended to June 3, 1944—a period of 11½ weeks. The average weekly number of men living in the oiled (2040) was approximately the same as those living in the unoiled (2015) blocks. During the period of observation there were 147 hospital admissions from the unoiled blocks, compared to 100 from the oiled blocks. The hospital admission diagnoses are given in table 3. The average weekly hospital admission rate from the unoiled blocks was 7.3, compared to 4.9 from the treated area. Although the

* In testing the statistical significance of observed differences for this paper the Student *t* test was applied. Because of evidence of non-normality in some of the samples, the sign test was also applied to the differences. It was found that the results of the two tests were very similar.

TABLE 2.—*Hospital admissions from oiled and unoiled blocks at Peterson Field February 28 to June 3, 1944.*

Week of admission:	Feb. 28–Mar. 6	Mar. 7–12	Mar. 13–19	Mar. 20–Apr. 2	Apr. 3–9	Apr. 10–22	Apr. 23–30	May 1–6	May 7–13	May 14–20	May 21–27	May 28–June 3	Total
Unoiled barracks	22	16	12	6	12	24	8	11	16	11	3	6	147
Oiled barracks	17	8	9	5	11	11	7	6	6	8	6	6	100
Difference	+5	+8	+3	+1	+1	+13	+1	+5	+10	+3	−3	0	47
	Weekly admission rate per 1000												Average
Unoiled barracks	10.9	7.9	6.0	3.0	6.0	11.9	4.0	5.5	7.9	5.5	1.5	3.0	6.1
Oiled barracks	8.3	3.9	4.4	2.5	5.4	5.4	3.4	2.9	2.9	3.9	2.9	2.9	4.1

Avg. weekly strength of unoiled blocks—2015, of oiled—2040.

incidence of disease is low these data are statistically significant in favor of the oiling procedures ($P<0.005$).

This investigation at Peterson Field was complicated by the fact that a sulfadiazine prophylaxis study against respiratory infections was superimposed on the only comparable groups, namely the combat training crews living in blocks 0 and 1. Here the difference was only slightly in favor of the oiled barracks. Other data not shown in tables 2 and 3 reveal that by taking additional areas as controls, differences varying from 9 to 30% were obtained in favor of the oiling measures. Thus no definite conclusion concerning the practical value of the dust suppressive measures can be drawn from the observations made at Peterson Field.

104th Infantry Division and artillery groups.—In the early part of March, this large infantry division, which had come to Camp Carson for final staging, moved into some of the oiled barracks previously occupied by the 71st Division. Additional barracks were then oiled to include essentially all the personnel in this group. In some areas every other barrack was oiled; in others, the barracks were oiled by company or battery. In some of the areas, oiled bedding was supplied. Observations on the effect of the oiling procedures began April 1 and continued until June 10, 1944.

TABLE 3.—*Hospital admissions with diagnosis from the unoiled and oiled blocks at Peterson Field, February 28 to June 3, 1944.*

Diagnosis	Unoiled blocks 1, 3, 6	Oiled blocks 0, 2, 4, 5
Nasopharyngitis	84	59
Tonsillitis	17	23
Scarlet fever	2	1
Rheumatic fever	3	1
Pneumonia	9	3
Bronchitis	5	4
Otitis media	7	1
Mumps	19	5
Measles	1	3
Total	147	100

Avg. weekly strength of unoiled blocks—2015, of oiled—2040.

Oiled floors alone.—The organizations in the 104th Division included the 104th Field Artillery Group, 339th Engineers, 804th Ordnance Company, Quartermaster Company, 415th Infantry Regiment, and approximately half of the 414th Infantry Regiment. The barracks housing the 104th Field Artillery Group were oiled by batteries, while those of other organizations were alternately oiled. Oiling was completed by March 20. The average weekly number of men living in the unoiled and oiled barracks was, respectively, 3818 and 3809. The personnel in these groups were considered to be seasoned troops, having been with the organization for six months to two years.

The total weekly admissions and admission rate per 1000 population from the oiled and unoiled barracks are shown in table 4. During the period of observation the weekly admission rates in the test and control groups varied but were consistently less in the former. The average weekly admission rate per 1000 from both the unoiled and oiled bar-

TABLE 4.—*Weekly admissions for respiratory diseases from barracks with unoiled and oiled floors at Camp Carson, April 1 to June 3, 1944.*

Week of admission:	Apr. 1–8	Apr. 9–15	Apr. 16–22	Apr. 23–29	Apr. 30–May 6	May 7–13	May 14–30	May 21–27	May 28–June 3	Total
Unoiled barracks	25	24	35	18	28	18	15	18	14	195
Oiled barracks	20	23	13	11	13	16	14	17	9	136
Difference	+5	+1	+22	+7	+15	+2	+1	+1	+5	59
	Weekly admission rate per 1000									Average
Unoiled barracks	6.6	6.3	9.2	4.7	7.3	4.7	3.9	4.7	3.6	5.7
Oiled barracks	5.3	6.0	3.4	2.9	3.4	4.2	3.7	4.5	2.4	4.0

Avg. weekly strength of unoiled barracks—3818, of oiled—3809.

racks were, respectively, 5.7 and 4.0. Again, although the weekly admission rates are low the data are statistically significant in favor of the oiled barracks ($P < 0.01$).

In this study group, as shown in table 5, approximately 81% of the admissions from the unoiled barracks and 79% of those from the oiled barracks were for nasopharyngitis. Approximately 58% of the nasopharyngitis cases for the control and treated group had throat cultures positive for B.H.S. (β-hemolytic streptococci). Likewise, the number of cases of measles and mumps admitted from the test and control groups was approximately the same.

TABLE 5.—*Hospital admissions with diagnosis from the unoiled and oiled barracks, April 1 to June 3, 1944.*

Diagnosis	Unoiled		Oiled	
	Number	Percent	Number	Percent
Nasopharyngitis (throats positive for B.H.S.)	92	47	62	46
Nasopharyngitis (throats negative for B.H.S.)	67	34	44	43
Otitis media	4		1	
Bronchitis	2		0	
Pneumonia	6		2	
Measles	10		12	
Mumps	14		15	
Total	195		136	

Test barracks: oiled floors only.
Avg. weekly strength of unoiled barracks—3818, of oiled—3809.

Alternate barracks with oiled floors and oiled bedding.—The recruits billeted in oiled barracks of approximately one-half the 414th Infantry Regiment and the 413th Artillery groups were also supplied with oiled bedding. The average weekly number of men living in the unoiled and oiled barracks was approximately the same, being, respectively, 1608 and 1616. The personnel consisted essentially of seasoned recruits. The total weekly admissions and admission rate per 1000 for the ten-week period of observation are shown in table 6. With the exception of the first week of observation there is a consistently and significantly lower rate of admission for the treated barracks. The average weekly admission rate per 1000 from the oiled barracks is approximately half that from the untreated controls, being respectively, 5.6 and 10.4. The over-all difference is statisti-

TABLE 6.—*Weekly admissions for respiratory and infectious diseases from unoiled floor barracks, and oiled floor barracks with oiled bedding, April 1 to June 10, 1944.*

Week of admission:	Apr. 1–8	Apr. 9–15	Apr. 16–22	Apr. 23–29	Apr. 30–May 6	May 7–13	May 14–20	May 21–27	May 28–June 3	June 4–10	Total
Unoiled barracks	14	15	27	26	14	23	15	12	8	13	167
Oiled barracks	16	12	16	12	4	9	9	7	2	4	91
Difference	−2	+3	+11	+14	+10	+14	+6	+5	+6	+9	76
	Weekly admission rate per 1000										Average
Unoiled barracks	8.7	9.3	16.8	16.2	8.7	14.3	9.3	7.5	5.0	8.1	10.4
Oiled barracks	9.9	7.4	9.9	7.4	2.4	5.6	5.6	4.3	1.2	2.5	5.6

Avg. weekly strength of unoiled barracks—1608, of oiled—1616.

cally significant ($P<0.01$) in favor of the oiling procedures.

As shown in table 7, the great majority of cases from the control (87%) and test (90%) barracks were admitted to the hospital with a diagnosis of nasopharyngitis, with or without throat cultures positive for B.H.S. Forty-six percent of the nasopharyngitis cases from the unoiled barracks had, on culture, throats positive for streptococci, while 57% of the throat cultures of similar cases from the oiled barracks were positive for this organism.

TABLE 7.—*Hospital admissions with diagnosis from the unoiled and oiled barracks April 1 to June 10, 1944.*

Diagnosis	Unoiled		Oiled	
	Number	Percent	Number	Percent
Nasopharyngitis (throats positive for B.H.S.)	67	40	47	51.7
Nasopharyngitis (throats negative for B.H.S.)	79	47	35	38.3
Rheumatic fever	1		1	
Bronchitis	2		1	
Pneumonia	4		4	
Measles	10		2	
Mumps	4		1	
Total	167		91	

Test barracks: oiled floors and oiled bedding, every other barrack, 413th Art. & 414th Inf. Reg.
Avg. weekly strength of unoiled barracks—1608, of oiled—1616.

286th Engineer Combat Battalion, oiled floors and oiled bedding by company.—This organization was activated at Camp Carson late in February. Oiling of the barracks floors and bedding was done by company from March 23 to 27, 1944. The personnel consisted essentially of new recruits with service records from two weeks to three months. Headquarters and C companies were oiled, and A and B companies served as controls. The average weekly number of recruits in the control and test groups was, respectively, 370 and 333. The observations on the incidence of infections before and after oiling barracks began March 5. The observations on the effect of the oiling procedures on hospital admissions, dispensary visits and streptococcal carrier rates covered the period from April 3 to July 30, 1944.

Hospital admissions and dispensary visits.—The hospital admissions and dispensary visits from the unoiled and oiled barracks are shown in figure 1 and table 8. During March and the first two weeks in April after the oiling was completed, there were more dispensary visits and hospital admissions for respiratory infections from the test than from the control barracks. Thereafter the weekly dispensary visits and hospital admissions from the unoiled barracks increased and remained signifi-

TABLE 8.—*Hospital admissions and dispensary visits of the 286th Engineer Combat Battalion April 3 to June 11, 1944. Barracks and bedding oiled by companies from March 24 to March 27, 1944.*

Week of admission:	Apr. 3–9	Apr. 10–16	Apr. 17–23	Apr. 24–30	May 1–7	May 8–14	May 15–21	May 22–28	May 29 June 4	June 5–11	Total
Dispensary visits											
Unoiled barracks	36	49	71	70	65	78	17	15	5	34	440
Oiled barracks	40	56	42	28	30	31	11	9	3	22	272
Difference	−4	−7	+29	+42	+35	+47	+6	+6	+2	+12	168
Dispensary visits—rate per 1000											Average
Unoiled barracks	97	132	192	189	176	211	46	41	14	92	119
Oiled barracks	120	168	126	84	90	93	33	27	9	66	82
Unoiled barracks	7	6	23	22	15	15	8	7	0	13	116
Oiled barracks	5	9	10	6	3	2	1	2	3	6	47
Difference	+2	−3	+13	+16	+12	+13	+7	+5	−3	+7	69
Hospital admissions—rate per 1000											Average
Unoiled barracks	19	16	62	60	41	41	22	19	0	35	31
Oiled barracks	15	27	30	18	9	6	3	6	9	18	14

Avg. weekly strength of unoiled barracks—370, of oiled—333.

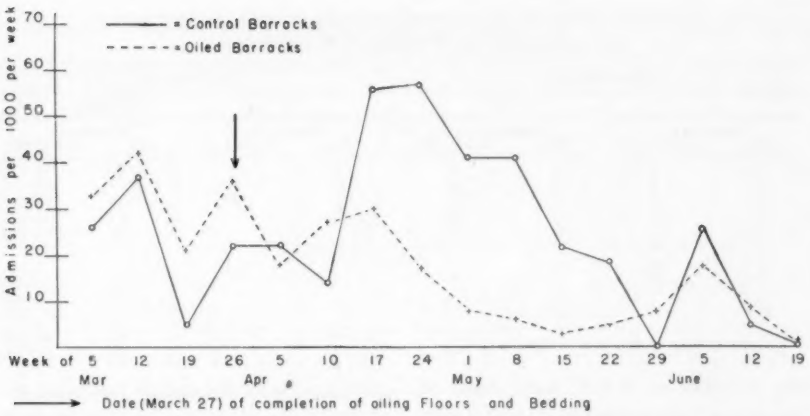

FIG. 1.—Comparison of hospital admission rates from test and control companies before and after application of oil to blankets and floors.

cantly higher throughout the period of observation (fig. 1, table 8). The average weekly dispensary visit rate per 1000 from the unoiled barracks was 119 compared to 82 from the oiled barracks. The average weekly hospital admission rates per 1000 from the control and test barracks were 31 and 14, respectively.

In table 9 are listed the admissions for the test and control barracks according to type of illness. Ninety-five in 89% of cases, respectively, admitted from the control and test barracks were for nasopharyngitis. Thirty-six and 37% of these, respectively, from the oiled and unoiled groups had throat cultures positive for streptococci. Although the study groups were small, the degree of difference in both dispensary visits ($P < 0.05$) and hospital admissions ($P < 0.01$) from the test barracks as compared with the control population suggests a definite effect of the oiling procedures.

The groups and types of streptococci found in the admission throat cultures from the control and test companies are shown in table 10. Types 3, 30 and 46 accounted for the majority of strains found in the throats of those admitted from control company A, while types 3 and 36 were the most prevalent types from control company B. Seven of the 11 types found in the throat cultures of test company C were group A type 3, while types 3 and 36 accounted for five of the six types found on throat culture in cases from Headquarters Company (table 10).

TABLE 9.—*Hospital admissions according to discharge diagnosis of patients admitted from the test and control barracks, April 1 to June 11, 1944.*

Diagnosis	Unoiled		Oiled	
	Number	Percent	Number	Percent
Nasopharyngitis (throats positive for B.H.S.)	43	37	17	36
Nasopharyngitis (throats negative for B.H.S.)	67	58	25	53
Rheumatic fever	1		1	
Otitis media	0		1	
Bronchitis	2		2	
Pneumonia	2		0	
Measles	0		1	
Mumps	0		0	
Meningitis	1		0	
Total	116		47	

Test barracks: oiled floors and oiled bedding, every other company, 286th Engineers.
Avg. weekly strength of unoiled barracks—370, of oiled—333.

Streptococcal carrier rates

Nose and throat culture surveys for

TABLE 10.—*Group and type of streptococci found in admission throat cultures from different companies, 286th Engineers.*

Control companies				*Test Companies*			
A		B		C		Hdqt.	
Streptococci		Streptococci		Streptococci		Streptococci	
No.	Group type	No.	Group type	No.	Group type	No.	Group type
1	1	1	1	1	1	2	3
5	3	8	3	7	3	1	17
1	6	1	28	1	36	3	36
1	17	5	36	2	A –		
1	19	1	46				
1	26	2	A –				
7	30						
1	33						
5	46						
2	Not A or C						
25		18		11		6	

A – = group A not typable with available serums.

the presence of B.H.S. were made on the barracks personnel on three different occasions, beginning approximately eight weeks after the test barracks had been oiled. From the findings listed in table 11 it is seen that oiling floors and blankets had no effect on lowering the carrier rates for hemolytic streptococci in the barracks' occupants. At the time of the first survey in May, approximately twice as many men living in the unoiled barracks carried streptococci in their throats as did those living in the oiled barracks. A month later (June) the rate had doubled in the test group. This was due principally to the marked increase in the number of carriers of types 3 and 17 streptococci. It should also be noted that these strains showed increased numbers in control company A, as seen in table 12. During this interval there were only a few hospital admissions, most of which were for non-streptococcal respiratory disease. Hospital admissions from both groups during July were likewise low, yet the throat carrier rates at the end of July were essentially the same and as high as were found during the June survey. Nasal carrier rates decreased markedly in the control group as well as in the group living in the oiled barracks between the end of June and the last of July.

It is interesting to note that the principal types of streptococci found in the hospital admission throat cultures of men from the different test and control companies as seen in table 10 are also the predominant types found in the respective company personnel during the various carrier surveys (table 12).

DISCUSSION

Since this study was completed, several reports have appeared concerning the use of dust suppressive measures alone and in combination with ultraviolet light for the control of respiratory tract infections in Army and Navy installations and in children's hospitals. Anderson and associates in England observed the effect of oiled floors alone on the

TABLE 11.—*Nose and throat carrier rates for B.H.S. in recruits billeted in oiled and unoiled barracks at Camp Carson, 1944.*

Date	Unoiled barracks			Oiled barracks		
	Number of men	Percent positive		Number of men	Percent positive	
		Throats	Noses		Throats	Noses
May 22–26	244	22.1	8.3	310	12.2	1.6
June 26–30	280	33.3	5.7	232	25.6	5.3
July 28–30	215	28.9	2.4	151	26.5	0.5

TABLE 12.—*Groups and types of streptococci found in throat cultures during carrier surveys of test and control companies of the 286th Engineer Battalion, 1944.*

Company date	Number cultured	A	C	Not A or C	A−	1	3	6	17	19	26	30	32	36	41	44	46	Others
							Test											
Hdq. co.																		
May 22	149	26	3	1	10	1	3	5						5		1		1
June 26	102	20	1	3	4	2	4	3						3			1	4
July 28	46	13		1	5		2	1						1			1	3
Co. C																		
May 25	161	12			7		3									2		
June 27	130	42			8	2	18	4	5					1		1		3
July 27	105	26	2		9		6	2	3		1			1			1	3
							Control											
Co. A																		
May 22	157	26	1	2	4	2					1	10	2		3		2	1
June 28	131	45		4	5		8	2	3	1	3	10		1			11	1
July 26	103	20	1	1	6				3		4	4					2	1
Co. B																		
May 24	87	24	2	1	4	4	7		1	1				5				2
June 30	149	48	3		8	5	19		3	1	1			8	1		1	1
Aug. 11	106	31	4	1	4	1	11		2	1	2			8			1	1

A− =group A not typable with available serums.

incidence of respiratory tract infections among Army personnel from December, 1942 to April 1, 1943.[5] Over this period of time there was a weekly infection rate of seven per 1000 population in the test unit compared to 38 in the control unit. Feasby and Bynoe,[6] over a similar period of time, observed a streptococcal disease rate of 77 in a control unit as compared to a rate of 18 in another unit, where living conditions were optimal as far as crowding and dust control was concerned. In two studies of the effect of dust control on the incidence of respiratory tract infections in Navy recruits reported by Miller and associates[7,8] and Shechmeister and colleagues[9] there was no consistent reduction in the incidence of disease in the oiled barracks. In the latter study[9] a slight reduction in the streptococcal carrier rates was observed in the group living in the oiled environments.

In the groups studied at Camp Carson the principal cause for admission was nasopharyngitis with or without throat cultures positive for hemolytic streptococci. The admission rates varied considerably. In all four groups there were fewer admissions from the oiled barracks. Only the observations made on the 71st Infantry Division were without statistical significance. In the three remaining groups where the effectiveness of oiled floors alone as well as oiled blankets and oiled floors was studied, the weekly hospital admission rates were of such magnitude that the differences in hospital admissions from the test and control areas were statistically significant in favor of the oiling procedures. In the case of the engineering battalion further evidence in favor of the

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9. Schechmeister, I. L. and Greenspan, F. S. 1947, The relation of the oil treatment of floors and bedding to the control of respiratory diseases among Naval personnel. Am. J. Hyg. **46**: 376–407.

dust control measures was the consistent reduction in dispensary visits as well as hospital admissions from the test barracks two weeks after the oil was applied, whereas for the five weeks preceding the oiling of the barracks there was no difference in the number of dispensary visits or hospital admissions from the test and control units.

These generally favorable observations made at Camp Carson are in contrast to those made during a subsequent joint study of the Commission on Acute Respiratory Diseases and the Commission on Air-Borne Infections at Fort Bragg, North Carolina, from October 1944 to May 1945.[10] During this study it was observed that during the period of low endemic occurrence of respiratory disease there was suggestive evidence that the oiling procedures reduced the incidence of hospitalized illnesses, but had no effect during the epidemic occurrence of acute undifferentiated respiratory disease. At Fort Bragg hemolytic streptococcal infections and other acute respiratory disease of bacterial origin did not occur with sufficient frequency to be used as criteria for evaluating the oiling procedures. In a naval training center, however, dust-suppressive measures alone were ineffective in reducing the severity of an epidemic of B.H.S. infections.[7,8]

The use of dust-suppressive measures for the control of streptococcal infections in children's wards has been studied by Wright and associates in England[11,12] and by Laurell in Sweden.[13] The first study by the English investigators[11] was definitely significant in favor of the oiling procedures. A highly communicable strain of streptococcus (type 6) was the principal cause of contamination of the ward environment and of cross infections. During the nine-week period of study, the cross infection rate was 18.6% on the test ward and 73.3% on the control, while the complications rate was only 2.8% on the test ward compared to 14.3% on the control. The following year in another hospital their results were negative.[12] This was interpreted as being due to the absence of a communicable strain of streptococcus and a consequent low rate of cross infection. In contrast to the first study where type 6 streptococcus was responsible for the great majority of infections on the wards, numerous types and groups of hemolytic streptococci were isolated from the cross infection cases during the second study. Laurell in Sweden[13] also observed no effect of oiling procedures on the incidence of cross infections due to B.H.S. in children's wards.

A review of previous studies shows that the application of oil to floors and bedclothes is now established as a most effective procedure in reducing the aerial contamination of barracks and ward environments.[14–17] However, the available

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data of the use of oiling procedures as a means of effectively controlling respiratory tract infections in barracks and hospital wards are equivocal. Although the results of the use of dust-suppressive measures at Camp Carson for the control of respiratory tract infections favor the oiling procedures, it cannot be assumed that oiling floors and bedding will bring about a similar effect in other environments, where the nature of the acute infections and modes of transmission may be different. On the other hand, where the incidence of streptococcal disease is high and where climate favors the accumulation of streptococci in the environment, dust control measures may serve more than simply as a measure of "good housekeeping."[28]

In intramural environments which are heavily contaminated with streptococci, such as was found at Camp Carson,[2,3] and in hospital wards[18–20] where patients with streptococcal disease are admitted, multiple opportunities exist for the spread of infection among the occupants. Direct contact with a heavily contaminated environment or with a "dangerous disperser"[19,21] who is dispensing large numbers of organisms may be equally as important as the inhalation of dust or air-borne organisms. The fact that a considerable number of streptococcal infections occurred in the oil treated barracks as indicated by the hospital admissions, as well as increase in the carrier rate among the occupants, emphasizes the importance of other modes of spread.

The control of hemolytic streptococcal infections and other acute respiratory diseases in military personnel continues to be an unsolved problem.[22,23] Methods, including dust control procedures, chemical disinfection with glycol vapor[24,25] and ultraviolet irradiation,[5,26,27] designed to keep the air free

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of bacteria have only a limited effectiveness and do not warrant widespread application as a means for controlling respiratory tract infections in military personnel. Of the three, oil to floors and bedclothes appears to the most practical for the suppression of dust-borne bacteria and should be a recommended procedure[28] in the over-all program of control of hospital cross infections[29,30] and infections among barrack personnel. The use of chemotherapeutic and antibiotic agents as a means of preventing the dispersion of pathogenic agents in the nose and throat secretions of "dangerous carriers" into the environment must also be considered in any program of respiratory disease control.[19,31]

SUMMARY

From January 1 to July 30 observations were made on the use of dust-suppressive measures for the control of acute respiratory tract infections among recruits at Camp Carson and Peterson Field, Colorado. The value of oiled floors alone and also in combination with oiled bedding as a means of preventing air-borne or dust-borne infections among new and seasoned recruits was studied. Simultaneous determinations of the bacterial content of the air in oiled and unoiled barracks showed that in the former there were over 90% fewer bacteria in the air during the periods of floor sweeping and bed making.[4]

The periods of observations on the incidence of infection in different groups varied from 5 to 13 weeks. In all groups studied there were fewer admissions from the oil-treated barracks than from the control barracks. With respect to oiled floors alone there was a difference in admissions of 15 and 30% in favor of the treated barracks. In the case of the 71st Infantry Division the observations, although in favor of the oiling procedures, are not significant. The observations made on the 104th Field Artillery group were, on the other hand, in favor of the dust-suppressive measures. The data gathered at Peterson Field on the use of both oiled floors and bedding were in favor of the oiled barracks and are of statistical significance.

In a third group of approximately 3200 seasoned recruits, one-half each living in oiled and unoiled barracks, there were average weekly admission rates, respectively, of 5.79 and 10.43 for the ten weeks of observation. This difference in rates is significant statistically in favor of the oiled barracks.

Likewise, in a smaller group of new recruits observed over a period of ten weeks, the average weekly hospital admission rate was 31 from the untreated and 14 from the oiled barracks. There was no difference or reduction in the streptococcal carrier rates in the men living in the test and control barracks.

In geographic areas where the incidence of streptococcal infections is high and where the climate favors the accumulation and survival of streptococci in the environment, dust-suppressive measures probably would be of significant value in preventing the dust-borne spread of this organism among the barrack occupants.

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ON THE MIGRATORY BEHAVIOR OF THE LARVAE OF VARIOUS ASCARIS SPECIES IN WHITE MICE

I. DISTRIBUTION OF LARVAE IN TISSUES

J. F. A. SPRENT

From the Department of Parasitology, Ontario Research Foundation, Toronto 5, Canada

Certain members of the family Ascarididae Blanchard, 1896* have already been shown to utilize one or two intermediate hosts during their life history. The adult stages of these parasites occur mostly in marine mammals (see Schwartz, 1925a), birds (Thomas, 1937) and fishes (Markowski, 1937). Walton (1937) has shown that ascaris parasites of certain snakes and alligators utilize amphibia as intermediate hosts, and Ortlepp (1922) demonstrated that the eggs of two ascaris parasites of the python hatch in the intestine of mice and migrate through their liver and lungs. It appears probable that moles and shrews may act as natural intermediate hosts for certain ascaris parasites of hawks and owls (Schwartz, 1925a; Chandler and Melvin, 1951). Although Fülleborn (1921b) suggested that certain ascaris parasites of terrestrial mammals may utilize intermediate hosts, this possibility has been somewhat overlooked since the discovery by Stewart (1917 and 1918a) of the life history of *Ascaris lumbricoides*. Stewart, having discovered the lung migration of the larvae in rodents, at first concluded that rodents act as intermediate hosts in the spread of this parasite, but later concluded (1918b, 1919, 1921) that only one host was used. The observations of Ransom and his coworkers (1917, 1919, 1920, 1921, 1922), Martin (1926), Roberts (1934) and de Boer (1935) have amply confirmed that pigs may become infected with *A. lumbricoides* directly by ingestion of embryonated eggs, the larvae migrating through the liver and lungs before they reach maturity in the intestine. Koino (1922) demonstrated that the human strain of *A. lumbricoides* makes a similar migration through the human body and confirmed earlier reports (see Martin, 1926) that human infection results from the ingestion of embryonated eggs.

There also seems to be little doubt that the life history of *Parascaris equorum* involves no intermediate host. Hadwen (1925) infected colts with the embryonated eggs of this parasite and later observed young ascaris in the duodenum. It seems likely that these larvae were derived from the experimental infection, and judging from the symptoms and lesions observed it was evident that the larvae migrated through the lungs and passed by way of the trachea and esophagus back to the intestine.

The mode of infection with *Neoascaris vitulorum* has been assumed by some to be similar to that of *A. lumbricoides*, although Brumpt (1936) failed to infect calves by feeding embryonated eggs from *N. vitulorum*, and the writer knows of no instance where successful

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* Parasites belonging to this family will be referred to as ascaris parasites.

experimental infection with this parasite has been achieved in the natural host. There are, however, numerous reports (Boulenger, 1922; Macfie, 1922, Schwartz, 1925 b; Vaidyanathan, 1949) suggesting that prenatal infection with this parasite occurs in calves. The author (1946) observed the eggs of *N. vitulorum* in the feces of a calf only 16 days old, a fact which can hardly be explained in any other way than by prenatal infection. The larvae of *N. vitulorum* were observed by Schwartz (1922) in the liver, lungs and kidneys of guinea pigs which had been fed embryonated eggs, but that rodents normally act as intermediate hosts of this ascaris species seems unlikely in view of the feeding habits of cattle.

The larvae of *Toxascaris leonina* do not regularly migrate through the liver and lungs of dogs, according to Wright (1935), but remain in the intestinal wall for several days and then return to the lumen where they reach maturity. Fülleborn (1922) observed encysted larvae in the mesenteric lymph nodes of experimentally infected guinea pigs and dogs.

In the case of *Toxocara canis*, both Petrov (1941) and Nifontov (1949) consider that prenatal infection is an important method of spread. They found a large proportion of stillborn fox cubs and puppies already infected with migrating larvae. The investigations of Fülleborn (1921b), Schillinger and Cram (1923), and Augustine (1927) were successful in establishing prenatal infection of puppies by administration of eggs to the bitch. No infection of the mother with mature parasites was established, although encapsulated ascaris larvae were found in the heart and lungs. Fülleborn (1921b) observed encysted living larvae in various tissues of rodents and dogs experimentally infected with eggs of *T. canis*.

Stewart (1918a) quoted several unsuccessful attempts by Davaine to infect kittens with the intestinal form of *Toxocara mystax* by feeding embryonated eggs. Sarles and Stoll (1935) have demonstrated the migration of larvae through the liver and lungs by feeding the eggs to kittens. It is doubtful whether any intestinal infections were established by these workers because the kittens were already naturally infected.

So far the only instance in which an intermediate host has been demonstrated in the spread of infection of the ascaris parasites of terrestrial mammals is that of *Ascaris columnaris*. After feeding the embryonated eggs to mice and other rodents Tiner (1949) observed encysted larvae in their tissues and successfully infected raccoons and skunks by feeding them with infected rodents. The present writer (1950) has repeatedly confirmed these observations insofar as the encapsulation of the larvae of *A. columnaris* is concerned, but has failed to obtain any experimental infection of raccoons or skunks.

This paper reports a series of experiments that were designed to reveal variations in the migratory behavior of the larvae of various ascaris species. The experiments involved the infection of white mice with the embryonated eggs and the counting of the larvae in different tissues at various intervals after infection. The mouse was chosen as an experimental animal because its organs are small enough to permit reasonably accurate counts and it could be obtained in a uniform size, weight, and age for all infections.

METHODS

Eggs of the various ascaris species were obtained from the uteri of the worms and were cultured in moist charcoal for 40 days at room temperature before being used. The adult worms were obtained from their various host species as

follows: *A. lumbricoides* was obtained from pigs at the abattoir, *A. columnaris* from the skunk (*Mephitis m. mephitis*), *Ascaris mustelarum** from the fisher (*Martes p. pennanti*), *T. leonina* from the lynx (*Lynx c. canadensis*) and the wolf (*Canis lupus lycaeon*), *Toxascaris transfuga* from the black bear (*Euarctos a. americanus*), *T. canis* from the dog and fox (*Vulpes fulva*), and *P. equorum* from the horse. The writer wishes to thank Dr. A. de Vos, Mr. C. D. Fowle and other members of the Department of Lands and Forests, Ontario, for their cooperation in supplying carcasses of wild animals. *N. vitulorum* from cattle and *A. lumbricoides* from man were obtained through the kind cooperation of Dr. R. L. Mayhew of the University of Louisiana and Dr. P. C. Beaver of Tulane University, respectively.

Infection of mice was performed by mixing the eggs, after an approximate count by dilution, with a little powdered meal in a shallow dish. The mice were deprived of other food for about 18 hours before the feeding of eggs and until after the contents of the dish were consumed. Eggs were administered in the afternoon and the meal was finished by the following morning. The mice used in these experiments were all males between 5 and 6 weeks old, and were obtained from the Connaught Laboratories of the University of Toronto. Most of them harbored two oxyurid nematode parasites in the caecum, *Syphacia sp.* and *Aspiculuris sp.*, but no nematode larvae were observed in the liver, lungs, intestinal wall or carcass of a random sample of 36 of these mice. The mice were killed by decapitation at the appropriate time after infection. Those designated as being killed "one day" after infection were killed in the morning about 42 hours after the infective meal was administered. The skin was removed, being discarded with the feet and tail; the liver, lungs and alimentary tract were removed for larval counts. The remainder of the mouse except for certain other tissues (see below) was designated as "the carcass" and ground in the Waring blendor for 45 seconds with 50 cc of saline. The emulsion was then mixed with 50 cc of saline containing 1 g of pepsin or trypsin. Whether pepsin or trypsin was used depended upon the time after infection that the mouse was killed. It was observed that the larvae of all of the species investigated were immobilized by peptic digestion if they were less than 8 days old. Their immobility resulted in some of them being carried to the surface of the digest and being lost during removal of the supernatant (see below). Tryptic digestion caused no disturbance of mobility even in the youngest larvae observed (24 hours) and also produced a more complete digestion so that the digest could be centrifuged before removal of the supernatant. On the other hand, trypsin was not suitable for the carcasses of mice killed later than 8 days after infection because by this time the larvae of some species had become encysted in the tissues, and while peptic digestion liberated the larvae from the cysts, trypsin failed to do so, with the result that encysted larvae were retained in the sieve. This did not preclude its use on the tissues other than the carcass, because the whole centrifuged digest was examined and encysted larvae could be readily counted. For these reasons the carcasses of mice killed up to 8 days after infection were digested with trypsin while the carcasses of those killed later than 8 days were digested with pepsin. If pepsin was used the pH of the mixture was adjusted to 1.0 with hydrochloric acid. After incubation at 37 C in a flask for 2 hours this emulsion was strained through a sieve (20 meshes per inch) and poured into a cylinder for sedimentation. After a further 2 hours all but 20 cc was removed by suction and the remainder was fixed with an equal volume of hot 6% formalin. When trypsin was used the method was the same as that described for pepsin except that the pH was adjusted to 7.0 with 0.1 *N* sodium hydroxide. After digestion with trypsin for 4 hours the digest was poured through a sieve and then centrifuged. Washing the material in the sieve was a more lengthy process than was necessary after pepsin, because trypsin did not clean the bones so thoroughly, about 3% of larvae being lost in this way. The liver and lungs were each ground with 25 cc of saline for 45 and 30 seconds, respectively, in the Waring blendor. After grinding, the emulsion was poured into a 50 cc pointed centrifuge tube containing 0.05 g of trypsin in 5 cc saline. The pH was adjusted to 7.0 and the tubes were incubated at 37 C for 3 to 4 hours, the tubes being periodically shaken. At the end of this time the tubes were centrifuged for 5 minutes and all but 10 cc was removed by suction. The remaining material was shaken and preserved by the addition of an equal volume of hot 6% formalin. The stomach, intestine, and caecum were removed and carefully freed of their contents firstly by allowing physiological saline solution to run through them and secondly by slitting them open and rinsing the surface. The washings from these operations were allowed to sediment and were preserved as "intestinal contents." The stomach, intestine and caecum were then ground together in the Waring blendor for 45 seconds with 25 cc of saline. They were subsequently treated in the

* The nomenclature of this parasite will be considered in a separate publication.

same manner as the liver and lungs and are referred to as "intestinal wall."

Each preparation, referred to respectively as carcass, liver, lung, intestinal contents and intestinal wall, was stored in a 10 cc screw-cap glass bottle. Larval counts were made by first removing all but 5 cc of the contents of the bottle after complete sedimentation. In the case of the intestinal wall it was occasionally necessary to retain 10 cc to avoid removing any of the sediment. The bottle was then thoroughly shaken, and 1 cc was quickly transferred with a pipette fitted with a bulb into a shallow glass dish which fitted the mechanical stage of the microscope. The total number of larvae occurring in half the dish were then counted, using a ruled eyepiece as a guide. In some instances there were too many larvae or the material was too opaque for this method of counting, in which case the volume was reduced to 3 cc and, after thoroughly shaking the bottle, 0.1 cc was removed with a pipette (a 1.0 cc graduated pipette with all but 2 divisions cut off). With practice it was possible to deliver 0.1 cc on to a slide with a minimum of delay to avoid any sedimentation either in the bottle or in the pipette. A coverslip was then applied to the drop on the slide. By the first method the number counted was multiplied by 10; by the second method three such counts were made and the average multiplied by 30.

The counts made of the larvae cannot be regarded as total counts for each mouse because certain organs were excluded. The head was discarded to avoid mixing of larvae which might be encapsulated in the tissues as a result of arterial distribution and larvae which might have migrated up into the nasal passages and sinuses from the lungs. Likewise the mesenteries, spleen and heart were not used for counting to avoid as far as possible mingling of larvae from different sources. In addition to these discarded tissues there were some tissues kept for histological examination, namely a piece of lung, liver, kidney and rectum. The sizes of these pieces were, as nearly as possible, the same for each mouse. Loss of larvae through digestion was estimated using liver and lung emulsions containing larvae of various species. The emulsions were mixed into three equal parts after emulsification. One part was digested with trypsin, another with pepsin and the third was not digested. Counts on these samples showed the superiority of the trypsin method in producing a more or less clear medium in which larvae were still mobile and thus easily and quickly counted. There was no significant difference in the total numbers of larvae in the three digests, though it was observed that a few larvae (about 0.1%) in the tryptic digest showed signs of partial digestion. It seems likely that while trypsin when used as described above does not harm the living larvae, it may partially digest the dead ones. Loss of small larvae by obliteration in the Waring blendor was estimated by comparing the number of larvae in teased brain tissue before and after the use of the blendor. The effect on larger larvae could be readily observed because the severed parts were observable. The indications were that errors in larval counts due to the use of the Waring blendor were negligible.

EXPERIMENTAL

Experimental work consisted of the infection of several series of mice with the eggs of different ascaris species. At daily intervals for 14 days after infection two mice were killed; at 21 and 28 days after infection three mice were killed; counts were made of the larvae recovered from the various organs. In this way an average count of the number of larvae occurring in a particular location of the mouse on different days following infection was obtained for several ascaris species. These counts are shown graphically in figures 1 to 8. For infection with *T. leonina* and *T. canis* 2500 eggs were given to the mice; for the remaining species 5000 eggs were given.

Ascaris lumbricoides

Figures 1 and 2 show that the larvae of the two strains of *A. lumbricoides* pass from the intestinal tract to the liver, where most of them remain for the first four days of infection. They do not become lodged in the wall of the intestine during their migration to the liver, although separate counts have shown that some of them do remain in the mesentery and associated tissues for the first three days. After four days the larvae began to leave the liver and appeared in the lungs in numbers which increased up to the 8th day. The lungs at this time showed severe and uniform congestion. After reaching the lungs the

larvae, according to Fülleborn (1921a and 1925), have two pathways which they may follow. One is to break through the alveolae and to travel up the trachea to the intestinal tract. In these experiments larvae appeared in the intestinal contents about five days after infection and continued to appear regularly for about a week. This migratory pathway will be referred to as the tracheal type of migration. The other pathway is to return to the heart from the lungs and to be distributed to various organs via the arterial system. This migratory pathway will be referred to as the somatic type of migration. The larvae appeared regularly in the carcass after about the fifth day of infection, although most of them had disappeared from the carcass by three weeks after infection. This indicates that they were either destroyed or reached some location whence they had access to the outside. None was observed to be alive after two weeks. It was evident that the majority of the larvae of both strains of *A. lumbricoides* follow the tracheal type of migration. The liver can be regarded in infection with this species as a reservoir where most of the larvae collect for the first four days of infection. After that time a flow of larvae commences to pass from the liver to the anus via the lungs, trachea and esophagus. The number of larvae recovered from the intestinal contents at any particular time probably depends upon the number of larvae in the liver at the beginning of the infection and upon the rate and duration of the migration. The occurrence of larvae in the intestinal wall probably indicates that some of them became temporarily enmeshed among the villi on their way out. Comparison between the distribution of larvae in mice infected with the pig and human strain of *A. lumbricoides* showed no significant difference in migratory behavior, but the human strain appeared to have about twice the infectivity.

Parascaris equorum

The migratory behavior of the larvae of this parasite (see fig. 3) resembled that of the larvae of *A. lumbricoides* from the pig. No larvae were retained in the intestinal wall before reaching the liver. The number of larvae in the lungs increased after the seventh day and rapidly decreased after the tenth day. Compared with *A. lumbricoides*, migration to the lungs appeared to be more gradual and at no time did the number of larvae in the lungs increase appreciably above the number recovered from the same animal's liver. This suggests that the outward flow was more steady and that there was less delay in the lungs. The larvae of this species followed almost entirely the tracheal type of migration, very few larvae appearing in the carcass and none remaining after four weeks.

Ascaris mustelarum and *Ascaris columnaris*

The larvae of *A. columnaris* and *A. mustelarum* can be considered together because their migratory behavior appeared to be very similar, though in marked contrast with that of *A. lumbricoides*. The most conspicuous characteristic (see fig. 4 and 5) was the more rapid and widespread migration of the larvae. On the first day after infection the larvae had reached the lungs and many of them had been distributed throughout the carcass. Mice were observed to show the most severe pulmonary symptoms on the third and fourth day of infection, at which time the lungs were uniformly dark red. While a few larvae appeared in the intestinal contents, possibly after a tracheal type of migration, most of them appeared to

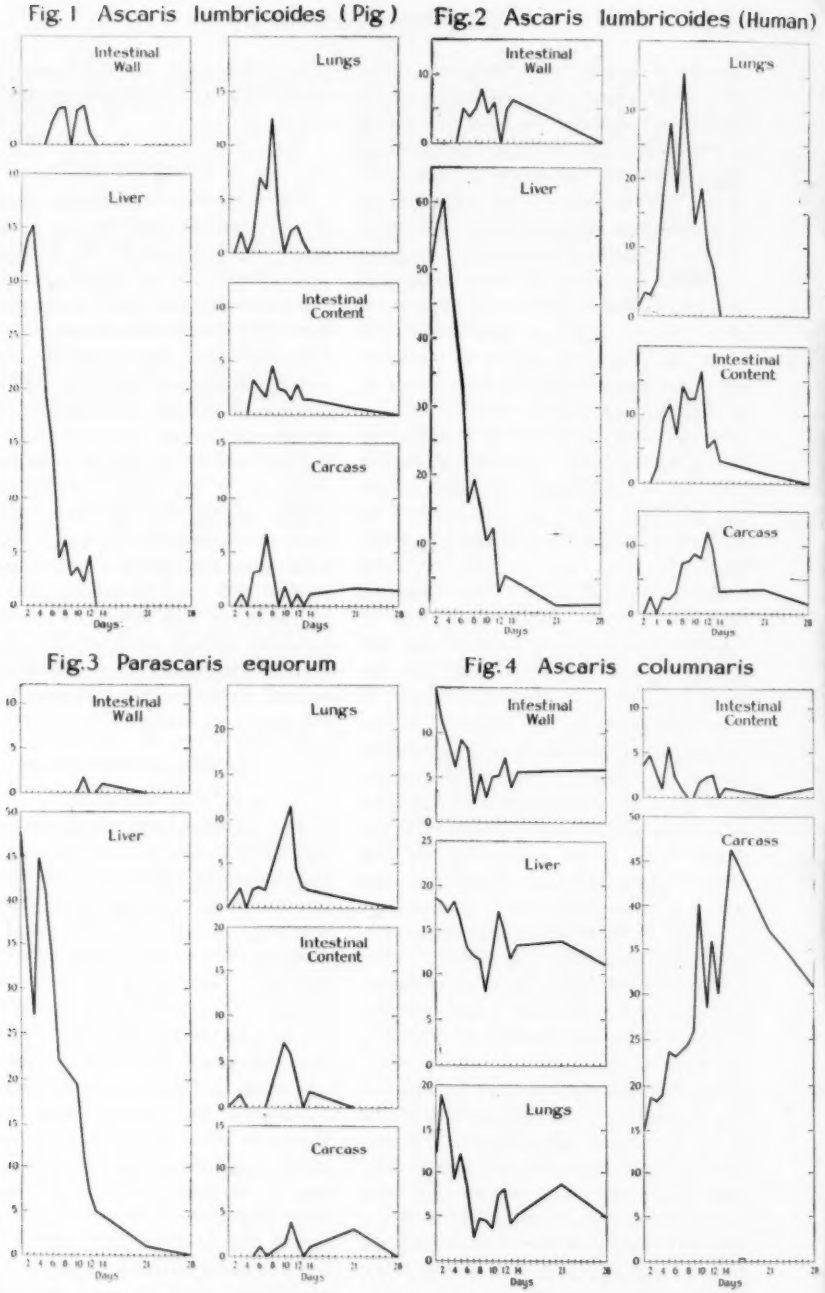


FIG. 1–8.—In these graphs the square root of the number of larvae counted in the intestinal wall,

Fig. 5 Ascaris mustelarum

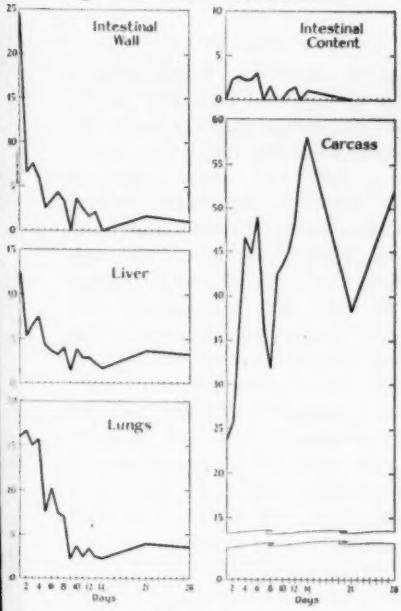


Fig. 6 Toxascaris leonina

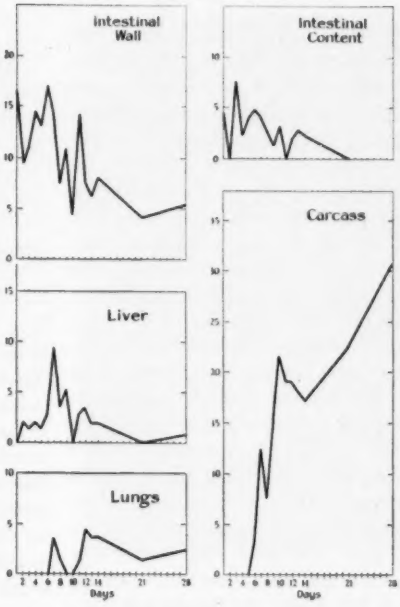


Fig. 7 Toxascaris transfuga

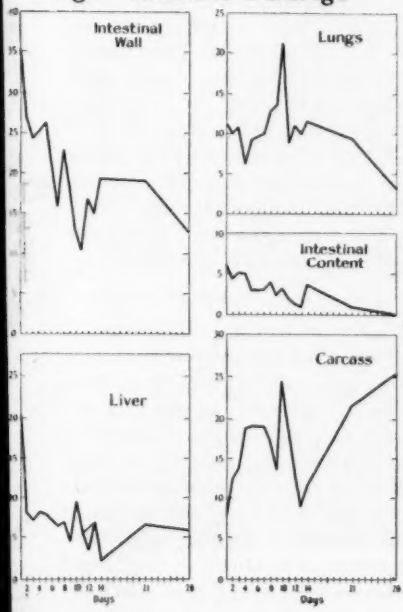
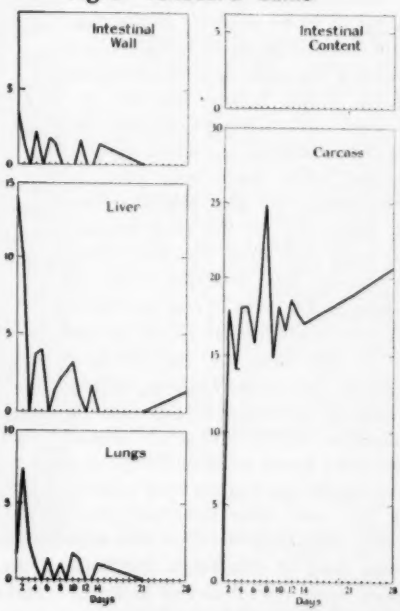


Fig. 8 Toxocara canis



liver, lungs, intestinal contents and carcass, respectively, has been plotted against duration of infection.

undergo the somatic type of migration, being distributed to the carcass. A relatively large number of larvae was recovered from the carcass between the fourth and the 28th day after infection. It therefore seems likely that the somatic migration occurs mostly within the first three days after infection. This would explain the early onset of pneumonia, several thousand larvae probably passing through the lungs within two or three days. Many larvae were recovered from the liver, lungs and intestinal wall throughout the whole course of infection with *A. columnaris*, but tended to become less numerous with the passage of time in the case of *A. mustelarum*. Some of these larvae may have remained from the initial migration through these organs, while others may have been distributed during the somatic migration after passage through the lungs. At about ten days after infection encapsulation of the larvae began to be evident; in the case of *A. columnaris* the heart, kidneys and liver showed the presence of white spots containing living larvae (see fig. 9). These were not so conspicuous in the case of *A. mustelarum*, in which species rectangular white spots were particularly numerous in the subcutaneous tissues, especially in the neck region, on the back and over the intercostal spaces (see fig. 10). Evidence of larval migrations in the form of red spots and streaks were visible, particularly on the surface of the skin, on the cerebral hemispheres and on the kidneys, within three days of infection in the case of both parasites. In the case of *A. columnaris* the mice began to show symptoms of injury to the central nervous system on the 17th day after infection; they frequently held their head on one side and some died at this stage. Nervous symptoms were not observed in infections other than *A. columnaris*.

Toxascaris leonina

Most of the larvae of this parasite (see fig. 6) appeared to remain in the wall of the intestine until the end of the first week, when the number of larvae recovered from the carcass began to increase and was maintained to the end of the experiment. The lungs had a distinctly mottled appearance on the sixth day after infection and this continued until 14 days after infection. Larvae were more frequently observed in the liver and lungs in the second week of infection. These observations indicate that the larvae of this parasite undergo a delayed somatic migration about a week after infection. Encapsulation of larvae was first evident at eight days after infection, particularly on the wall of the intestine and caecum, but also on the subcutaneous tissues of the neck, back and intercostal region. At 11 days the number of larvae in the intestinal wall began to decline, many of the cysts being empty. This can probably be explained by the migration of the larvae into the intestinal lumen and their elimination in the feces. It would appear that these results confirm those of Wright (1935), the larvae of this species showing a tendency to remain in the intestinal wall. However, a considerable number of larvae reached the carcass and were still alive after 28 days.

Toxascaris transfuga

The larvae of this species (see fig. 7) showed a marked tendency to remain in the intestinal wall, resembling in this respect the larvae of *T. leonina*. A considerable number of larvae had evidently migrated to the lungs by the first day, at which time the lungs showed red spots and patches which increased to a uniform congestion by the third day. The appearance of larvae in the lungs and carcass on the first day of infection indicated that somatic migration oc-

curred earlier with *T. transfuga* than with *T. leonina*. The larvae observed in the contents of the intestine during the first week of infection were probably those which had undergone a tracheal migration, while those appearing later were probably partly derived from the intestinal wall. Encapsulation of larvae on the intestinal wall was first observed at eight days. After this time encapsulated living larvae were observed regularly on the rectum, caecum and in the subcutaneous tissues of the neck, back and intercostal regions (see fig. 11 and 12).

Toxocara canis

The migration of the larvae of *T. canis* (see fig. 8) resemble those manifested by *A. columnaris* in that the larvae appear in the carcass relatively early in the infection. They differ in the absence of larvae from the intestinal contents, which probably indicates an exclusively somatic type of migration. They differ also in the early disappearance of larvae from the intestine, liver and lungs. The kidneys were particularly severely affected by this species, red streaks and spots becoming evident on the surface at about three days after infection. Separate counts of the larvae in the kidneys showed that the larvae enter the kidneys within two days of infection but have mostly left them by the seventh day. Encapsulated larvae were first observed on the 12th day after infection. They occurred most frequently on the subcutaneous tissues of the back, legs and chest.

FIG. 9.—Encapsulated larvae of *A. columnaris* in heart and liver of mouse at six weeks after infection.

FIG. 10.—Encapsulated larvae of *A. mustelarum* in musculature of mouse at six weeks after infection.

Neoascaris vitulorum

Sufficient eggs of this parasite were not available. Further investigation is necessary before any conclusions are made regarding its migratory behavior.

FIG. 11.—Encapsulated larvae of *T. transfuga* in caecum of mouse at four months after infection.

DISCUSSION

The results recorded above demonstrate that the various ascaris species investigated can be grouped into two categories depending upon the migratory behavior of the larvae. The first category contains those species whose larval migrations lead to temporary infection of the rodent. The second category contains those whose larval migrations lead to semipermanent or perhaps permanent infection of the rodent. In the first category may be placed both human and porcine strains of *A. lumbricoides* and also *P. equorum*. Figures 1 to 3 show that 28 days after infection the mice were practically free of larvae. The larvae had migrated to the liver and lungs and then, after a tracheal type of migration, were eliminated via the intestine. These results are in accordance with the view that with these species infection of the rodent and the migrations of the larvae in the rodent are accidental, the larvae behaving in the rodent as they do in the definitive host. The second category contains *A. columnaris*. *A. mustelarum*, *T. canis* *T. leonina*, and *T. transfuga*. The mice infected with the larvae of these parasites all showed at 28 days after infection the presence of living larvae encapsulated within various tissues. In the case of both *Toxascaris* spp. many larvae were observed in the intestinal wall, while in the other species they were mostly distributed among the somatic tissues.

It is necessary at this point to consider the significance of encapsulated larvae in the life history of these parasites. Three interpretations could be used to explain their presence. The first interpretation is that the behavior of the larvae in the rodent is of no significance in the life history of the parasite because

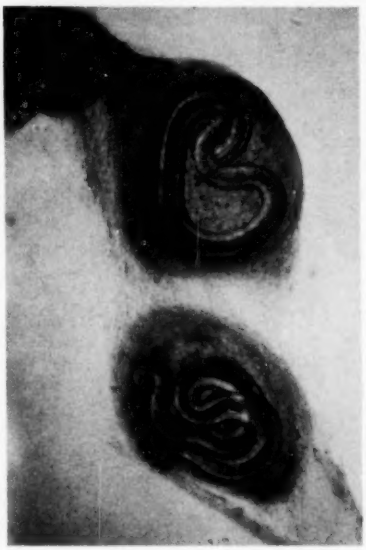


FIG. 12.—Encapsulated larvae of *T. transfuga* from intercostal muscles of mouse four months after infection. ×57.

it results from accidental entry into an abnormal environment. In other words, if the larvae had gained access to the definitive host they would have undergone a migration which would have led to intestinal infection rather than somatic infection. The second interpretation is that the behavior of the larvae in the mouse may be a definite adaptation towards utilization of the rodent as a true intermediate host. The third interpretation is that the larvae in the mouse are behaving in the same manner as they do in the definitive host.

The first interpretation would appear to be feasible if it were proved that natural infection of the definitive host occurred through the ingestion of embryonated eggs, and that the larvae undergo a tracheal type of migration in the definitive host, resulting in intestinal infection in the manner described for *A. lumbricoides*. This in the opinion of the writer has not been conclusively proved for any of the ascaris parasites of carnivores.

In support of the second interpretation are instances where experimental intestinal infection of the definitive host has been achieved through the ingestion of a rodent harboring encapsulated larvae. Tiner's (1949) results, if confirmed, will be strong support for this interpretation in the case of *A. columnaris*. For the following additional reasons it appears likely that rodents act as true intermediate hosts in the life history of *A. columnaris* and *A. mustelarum*. Firstly, it was observed in the case of *A. mustelarum* that about four-fifths of the larvae ingested as eggs made their way to the somatic tissues of the mouse. If a similar migration were to occur in the definitive host, it is evident that only a fifth of the larvae ingested could return to the intestinal lumen. All other larvae could be encapsulated unless they entered fetal tissues. As immature forms of these species are frequently encountered in adult animals it seems unlikely that prenatal infection is the regular method of infection. Secondly, in the present writer's experience (unpublished) the eggs of these species are noninfective to their definitive hosts. Whether natural infection with *Toxocara* spp. and *Toxascaris* spp. can occur through ingestion of encapsulated larvae from rodents awaits further experimentation. Nevertheless, the presence of living larvae in the tissues of mice indicates at least that temporary intestinal infection with these species must take place if the definitive host eats infected mice. Fülleborn (1921b) recovered larvae of *T. canis* from the lungs of a dog which had swallowed encysted larvae.

The third interpretation, namely that the behavior in the mouse is similar to that occurring in the definitive host, is strongly supported in the case of *T. leonina* and *T. transfuga*. The encapsulation of the larvae in the intestinal wall of the mouse appears to resemble that described by Wright (1935) as occurring in the dog. In this case the encapsulated larvae are able to return to the intestinal lumen and thus direct infection by eggs is made possible. It seems likely that this third interpretation fits the case of *T. canis* and possibly also *N. vitulorum*. With both these parasites prenatal infection appears to be the usual mode of spread (see Introduction). If an exclusively somatic type of migration occurred in the definitive host as was observed in the mouse infected with *T. canis*, larvae would not reach the intestinal lumen of the animal ingesting the eggs, but would, among other tissues, be distributed to the placenta, whence they could reach the fetal liver and lungs. In this way prenatal infection would become the only method of infection.

SUMMARY

Mice were infected by feeding the embryonated eggs of the following ascaris species: *Ascaris lumbricoides* (pig), *Ascaris lumbricoides* (human), *Ascaris columnaris*, *Ascaris mustelarum*, *Parascaris equorum*, *Toxascaris leonina*, *Toxascaris transfuga* and *Toxocara canis*. The distribution of the larvae in the various tissues of the mice at daily intervals from one to fourteen days as well as at three and four weeks after infection was investigated.

Two kinds of migratory behavior were observed. The first was manifested by most of the larvae of *A. lumbricoides* and *P. equorum* and consisted of migration through the liver, lungs and intestine, followed by eventual disappearance of living larvae from the tissues of the mouse (tracheal migration). The second type of migratory behavior was manifested by the larvae of the other species investigated, and resulted in more or less permanent infection of the rodent with encysted but living larvae (somatic migration). While the encysted larvae of *T. canis*, *A. columnaris* and *A. mustelarum* occurred mostly in the subcutaneous connective tissue and in tissues other than the intestines, many of the larvae of *T. leonina* and *T. transfuga* were encysted in the wall of the caecum and rectum.

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THE ENHANCEMENT OF THE VIRULENCE OF *CANDIDA ALBICANS* IN MICE

S. B. SALVIN, J. C. CORY, AND MARGARET K. BERG

From the Rocky Mountain Laboratory, Hamilton, Montana (National Microbiological Institute, National Institutes of Health, Public Health Service)

One of the difficulties in the study of therapy in moniliasis is the absence of a small laboratory animal that is readily killed by the etiologic organism, *Candida albicans*. Although the rabbit is extremely susceptible to infection, the use of the rabbit introduces problems relating to space, expense, and availability, where large numbers of animals are necessary. Therefore, attempts were made to produce high rates of infection and mortality in a small laboratory animal. The present report shows that uniformly successful results can be obtained in the white Swiss mouse (Rocky Mountain Laboratory strain).

MATERIALS AND METHODS

Four isolates of *C. albicans* were used: 3148 (from the collection at National Institutes of Health) had been obtained from a case of endocarditis; 3160,* from the American Type Culture Collection; 3161* from an adult case of thrush; and 3162* from a normal throat. The cultures were maintained on agar slants containing 2% dextrose and 1% Difco Neopeptone. The inoculum was prepared from 24- to 48-hour cultures of the yeast phase grown at 37 C in a broth composed of Difco casamino acids, 10 g; yeast extract, 1.8 g; NaCl, 1.5 g; KCl, 1.5 g; Na_2HPO_4, 1.5 g; dextrose, 2 g; and distilled water to make 1000 ml. One hundred and fifty ml of broth were dispensed in 500-ml flasks and kept in constant motion on a rotary shaker during the incubation period of 24 to 48 hours. The number of cells was determined by means of a Levy hemacytometer. The number of viable cells was not determined by plate count because the cells form small groups, making such counts inaccurate.

The 5% gastric mucin was prepared as follows: granular mucin (type 1701-W, Wilson Laboratories) was suspended by a Waring blendor to a concentration of 5% in distilled water. After standing for about 48 hours, the pH was adjusted to 7.2 with sodium hydroxide and the resulting suspension filtered through fine cheesecloth. The mucin suspension was then sterilized by heating to 100 C for three 30-minute periods within 24 hours. The 5% mucin was then injected into mice with an equal amount of cells in saline.

Sixty-day-old mice were used throughout, except for the experiments in which the influence of age on mortality was studied. Their average weight was 26.5 g for the males and 21.3 g for the females. Cultures of the kidney and liver were made from the mice that died spontaneously on or before the 30th day after inoculation and incubated at room temperature.

RESULTS

The number of fatalities in mice inoculated intraperitoneally with suspensions of the yeast cells of *C. albicans* in mucin was much greater than the number of fatalities in the mice inoculated similarly with saline suspensions (table 1).

TABLE 1.—*Effect of mucin on virulence of isolate 3148 of* C. albicans *in 60-day-old mice (deaths within 30 days after inoculation).*

Exp. no.*	Dose per injection $\times 10^6$ (in 0.5 ml)	Number of deaths in mice inoculated with cells in	
		Saline	2.5% mucin
I	22	0/6†	5/6
	7.3	0/6	5/6
	2.4	0/6	2/6
II	22	0/12	7/12
III	21	0/6	6/6
	5	0/6	5/6
	1.25	0/6	3/6
	0 (controls)	0/6	0/13

* These experiments extended over a period of six months. Both male and female mice were used, but in similar quantities in the groups with and without mucin.

† In this and other tables the numerator indicates the number of deaths and the denominator the total number of mice in a particular experimental group.

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* Obtained from Dr. C. H. Drake, Washington State College, Pullman, Wash.

TABLE 2.—*Relationship of size of dose of isolate 3148* (C. albicans) *in 2.5% mucin and mortality of 60-day-old Swiss mice.*†

Total dose per mouse* ($\times 10^6$)	Mortality in mice	Percent
198	57/57	100
99	14/14	100
66	33/38	87
49.5	25/38	66
22	23/35	66
16.5	7/24	29
11	7/29	24

* The dose was mixed with an equal quantity of 5% mucin, and 0.5 ml was injected intraperitoneally into each mouse.
† Males and females were used in comparable quantities.

In the groups of animals inoculated with over a million organisms in mucin, one or more animals died before the 30th day after injection. However, of the 48 mice inoculated with doses of 1.25 $\times 10^6$ to 22$\times 10^6$ in saline, no mice died, in contrast to the 33 (69%) that died when the inoculum was suspended in 2.5% mucin.

When very large doses were used, death also occurred among the mice that were inoculated with the fungus cells suspended in saline. For example, 14 of 14 mice died when injected intraperitoneally with 198$\times 10^6$ cells in mucin or in saline. However, all of the mice that were inoculated with cells in mucin were dead by the start of the third day, whereas the mice that were inoculated with cells in saline died from the third to the 19th day.

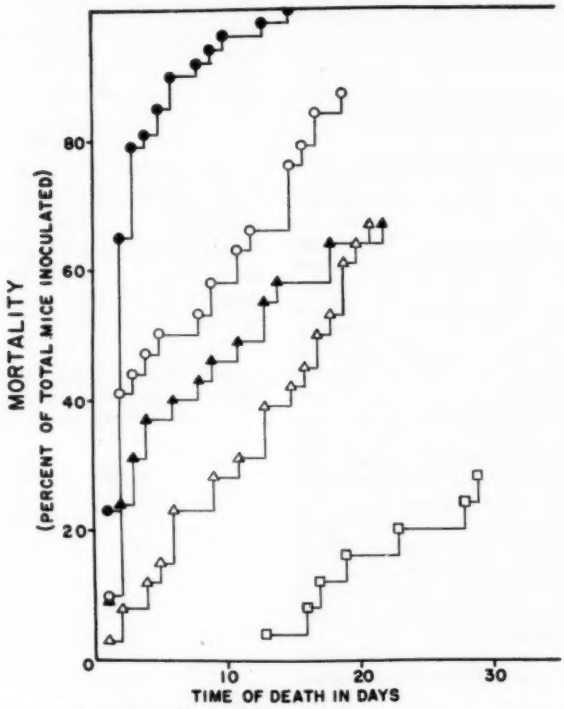

FIG. 1.—Cumulative death rates for 60-day-old Swiss mice within 30 days after infection with varying doses of the yeast cells of isolate 3148 of *C. albicans*. Solid circle = 198$\times 10^6$ cells, circle = 66 $\times 10^6$ cells, solid triangle = 49.5$\times 10^6$ cells, triangle = 22$\times 10^6$ cells, square = 16.5$\times 10^6$ cells.

Examination of the death rates of 60-day-old mice inoculated intraperitoneally with varying doses of cells in 2.5% mucin indicates that for isolate 3148 the LD_{100} was about 99×10^6 cells and the LD_{50} slightly below 22×10^6 cells (table 2). The time of death after infection also varied greatly according to the number of cells in the inoculating dose (fig. 1), with the higher doses causing earlier deaths.

The kidneys of all animals that died were cultured on agar slants, and with the exception of two that were bacterially contaminated, all yielded growth of *C. albicans*. Gross lesions were frequently seen on these kidneys and occasionally on the liver and intestines.

Examination of the susceptibility of mice of different ages to suspensions of isolate 3148 in 2.5% mucin revealed marked differences (table 3). The pooled results of all experiments for a single age showed that 17 of 18 of the three-week-old mice died within 30 days after infection. The younger mice also died at a faster rate at a given dose, and with a smaller inoculum than the older ones. Six of six three-week-old mice died within 48 hours after a dose of 69×10^6 cells, while the fifth and last of six 13-week-old mice died 21 days after infection.

Sex, however, had no marked influence on the susceptibility of 60-day-old mice to infection. Of 195 males and 180 females inoculated intraperitoneally at various times with equivalent doses of cells in mucin, mortality was almost the same, namely, 51.8% for the males and 46.1% for the females. The rate at which the two sexes died seemed somewhat similar (fig. 2), except for a slight tendency of the females to die at a faster rate during the first week after inoculation.

C. albicans was recovered in culture from the kidneys of each mouse that died, with the exception of five. Of these,

TABLE 3.—*Mortality figures on white Swiss mice of six different ages injected intraperitoneally with one of three suspensions of the yeast phase of isolate 3148 in 2.5% mucin.*

Age*	Inoculated with 69×10^6 cells						Inoculated with 23×10^6 cells						Inoculated with 7.7×10^6 cells						Total dead
		Time of death						Time of death						Time of death					
	Total	1st wk.	2nd wk.	3rd wk.	4th wk.	Survivors†	Total	1st wk.	2nd wk.	3rd wk.	4th wk.	Survivors†	Total	1st wk.	2nd wk.	3rd wk.	4th wk.	Survivors†	
3 wk.	6	6	0	0	0	0	6	6	1	0	0	0	6	2	2	1	0	1	17/18
5 wk.	6	4	1	0	0	0	6	1	1	0	0	4	6	0	0	2	0	4	10/18
7 wk.	6	3	3	0	0	0	6	0	1	0	0	5	6	0	1	0	0	5	8/18
9 wk.	6	2	1	1	0	2	6	1	2	1	0	4	6	0	0	0	0	6	6/18
11 wk.	6	3	2	1	0	0	6	0	0	1	0	5	6	0	0	0	0	6	7/18
13 wk.	6	2	2	1	0	1	6	0	0	1	0	5	6	0	0	0	0	6	6/18
Total	36					3/36	36					23/36	36					28/36	

* Age of animals at time of inoculation.
† Survivors after 30th day postinoculation.

cultures from four were bacterially contaminated, and one was negative. Gross lesions were observed on the kidneys of 24 of 139 mice.

Series of female mice were also infected by different routes, namely, intraperitoneal, subcutaneous, intracerebral, and intercardial, and the resulting mortalities compared (table 4).

Marked differences in death rates were observed, depending on the route of inoculation. After the pooling of the results of all experiments for a single route of infection, it was obvious that inoculation by the intracerebral route produced the highest percentage of deaths. Injection by the subcutaneous route, on the other hand, induced almost no fatalities. Introduction of the fungus cells by the intraperitoneal or intracardial routes resulted in an intermediate number of deaths.

Not only were there marked differences in the percentages of deaths, according to the inoculation route, but also the time by which a given percentage of animals was dead varied greatly. For example, of the 60 animals infected intracerebrally, all were dead seven days later, with 65% having died during the first 24 hours postinoculation. Fifty percent of the mice injected intraperitoneally were dead by the fifth day. When the mice were injected intracardially 50% were also dead by the second day. Of interest is the fact that the two deaths among the mice infected subcutaneously occurred within 48 hours after infection.

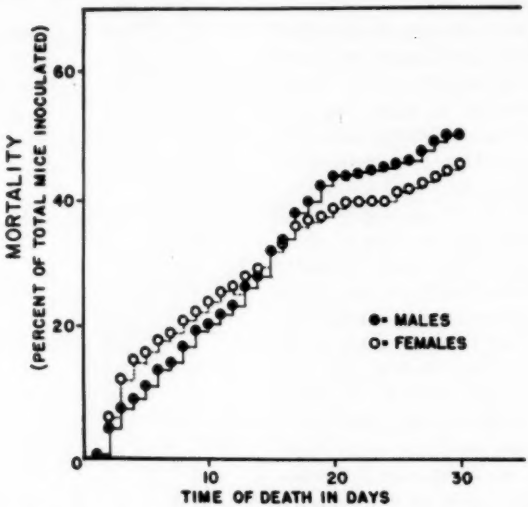

FIG. 2.—Cumulative death rates among male and female mice within 30 days after infection with doses of 5.5×10^6 to 198×10^6 yeast cells of isolate 3148 of *C. albicans*.

The virulence of four strains of *C. albicans* was then compared by intraperitoneal inoculation of fungus cells in 2.5 mucin (table 5). It is evident that the percentage of mice that died from any given dose depended on the particular strain used. For example, an intraperitoneal injection of 66×10^6 cells in 2.5% mucin caused the death of 23 of 28 mice (82%) for isolate 3148, eight of 28 (29%) for isolate 3160, 28 of 28 (100%) for each of isolates 3161 and 3162.

There was also a marked difference in

the time by which any given percentage of mice was dead due to a specified dose. For example, when the mice were infected with 66×10^6 organisms of isolate 3148, 50% of the mice had succumbed by the 11th day. A similar dose of 3160 killed only 29% of the mice. A similar inoculation of either isolate 3161 or 3162 produced death of 50% of those inoculated early on the third day. When the time of death due to inoculation of tions be rigidly controlled. This applies not only to the inoculum, which should be grown with continuous agitation for 24 to 48 hours in a satisfactory medium after transfer from a stock culture, but also to the mice, which must be of a suitable age and sex. The use of 2.5% mucin as a vehicle for injecting the fungus cells has made possible the employment of a relatively small number of cells to produce a high percentage of

TABLE 4.—*Comparison of mortalities of 60-day-old mice injected with one of three doses of yeast cells of* C. albicans *in 2.5% mucin by one of four specified routes.*

Dose of injection $\times 10^6$	Intra-peritoneal	Subcu-taneous	Intra-cerebral	Intra-cardial
66	19/20	0/20	20/20	8/17
22	15/20	2/10	20/20	18/20
7.3	5/20	0/20	20/20	17/19
Total	39/60 (65%)	2/50 (4%)	60/60 (100%)	43/56 (77%)
0 (mucin alone)	0/12	0/12	2/12	1/12

TABLE 5.—*Comparative death rates of 60-day-old mice within 30 days of an intraperitoneal inoculation with varying doses of one of four isolates of* C. albicans.

Dose ($\times 10^6$)	Number and (%) deaths due to isolate:			
	3148	3160	3161	3162
198	28/28 (100)	17/28 (61)	28/28 (100)	28/28 (100)
66	23/28 (82)	8/28 (29)	28/28 (100)	28/28 (100)
44	9/14 (64)	1/14 (7)	14/14 (100)	10/14 (71)
22	9/28 (32)	1/14 (7)	24/28 (86)	25/28 (89)
11			17/28 (61)	12/28 (43)
5.5	1/14 (7)	0/14 (0)	4/14 (29)	6/14 (43)
Mucin	0/14	0/14	0/14	0/14

isolate 3161 or 3162 was compared at a weaker dose level, such as 22×10^6 cells, it was found that 50% of those infected with 3161 were dead by the 15th day; with 3162, by the 13th day.

Thus, isolate 3160 was a comparatively avirulent strain, 3148 somewhat more virulent, and 3161 and 3162 the most virulent and about equally so. This virulence was indicated both by the time of the deaths and the number of deaths due to a given dose.

DISCUSSION

The preceding experiments indicated that a base line for the determination of the virulence of *C. albicans* in white Swiss mice can be obtained. However, it is essential that experimental condi- fatalities. By varying the number of organisms inoculated in mucin, the route of injection and the age of the mice, the death rate and the time lapse between infection and death could be controlled. Experiments which heretofore had been limited by the relative resistance of small laboratory animals (e.g., mouse, rat, and guinea pig) to infection by *C. albicans* now may be carried out because of the virulence enhancement by the mucin. This action of mucin with *C. albicans* is seemingly similar to that of mucin with *Histoplasma capsulatum*.[1]

1. Campbell, C. C. and Saslaw, S. 1950, Use of mucin in experimental infections of mice with *Histoplasma capsulatum*. Proc. Soc. Exper. Biol. & Med. **73**: 469–472.

SUMMARY

1. In a series of experiments over a six-month period, 24 to 100% of white Swiss mice died within 30 days after being injected intraperitoneally with doses of 1.25×10^8 to 22×10^6 yeast cells of *Candida albicans* when suspended in 2.5% mucin. No mice died when inoculated with similar numbers of cells in saline.

2. Twenty-one-day-old mice were the most susceptible of the six age groups tested (three to 13 weeks).

3. There was no marked difference in susceptibility between male and female mice of sixty days' age after intraperitoneal infection with the organism in mucin.

4. Of four infection routes studied, the intracerebral one produced the most fatalities and the subcutaneous route the least fatalities. Inoculation by the intraperitoneal or intracardial routes was intermediate in its effect.

5. Four isolates showed distinct differences in their virulence in 60-day-old mice.

During the preparation of this paper, the publication entitled "The use of gastric mucin to lower resistance of laboratory animals to systemic fungus infections," by Richard E. Strauss and Albert M. Kligman, appeared in the Journal of Infectious Diseases, **88**: 151–155. These authors reported 28 of 30 mice died after infection with *C. albicans* in gastric mucin. None of 30 control animals died within 14 days after inoculation. No further data are given.

PROPHYLAXIS OF GERMAN MEASLES WITH IMMUNE SERUM GLOBULIN

ROBERT F. KORNS

From the Bureau of Epidemiology and Communicable Disease Control, New York State Department of Health, Albany, New York

Ever since the work by Gregg,[1] which called attention to the significance of German measles as a cause of congenital defects when the disease attacks mothers during the first trimester of pregnancy, concern has been expressed about the means for avoiding this eventuality. Actually, the exact risk involved, in terms of the percentage of such pregnancies which end in the birth of a defective child, is not known. However, based on the reported evidence,[2–4] there is no doubt that a relationship exists between the appearance of certain congenital defects and the occurrence of German measles in the mother early in pregnancy. This association has appeared so striking to some authors that they have advised therapeutic abortion in instances where pregnant women have acquired the disease early in pregnancy. Others have urged that young girls be exposed deliberately to the disease early in life for the purpose of acquiring persistent immunity.[5] Finally, many have suggested that exposed pregnant women be passively immunized with convalescent serum or immune serum globulin. Several attempts have been made to evaluate this procedure but with inconclusive results, largely because of the small size of the groups involved[6,7] or because of inadequately controlled observations.[8] One report[9] is more impressive than others and indicates very adequate protection afforded by immune globulin during the period 4 to 18 days after inoculation. The present study was initiated in order to evaluate further the prophylactic use of immune globulin.

A situation appropriate for study was discovered at the Rome State School in Rome, New York, an institution housing 4113 mentally defective patients. The first discovered case of German measles had onset on July 24, 1948, and from that time, the disease existed almost continuously in the population until May, 1949, a total of 410 cases having been reported. It smoldered in endemic fashion in several buildings, occurring in explosive outbreak fashion from time to time as new buildings became involved. During this period of nine months, the attack rate by age groups was as follows: 0 to 9 years, 38.9%; 10 to 14 years, 20.9%; 15 to 19 years, 11.0%; 20 years, 1.9%. Actually, 67% of the population of the

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It is a pleasure to acknowledge the assistance of the staff of the Rome State School, Dr. James P. Kelleher, Director, in making these studies possible.

Special mention should be made of the help furnished by Mr. George Trustan, Mr. Noel Smith and Dr. Anne M. Bahlke in carrying out the first study, and to Dr. Evelyn Rogers for her help in the second study.

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2. Swan, C. and Tostwin, A. L. 1946, M. J. Australia, p. 645.
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TABLE 1.—*German measles at Rome State School. Age distribution of immunized and control groups and the cases developing during the 20-day period following administration of immune serum globulin, lot no. 8214-2. First study.*

Age	Population		Cases of German measles	
	Treated	Controls	Treated	Controls
Under 5	14	8	0	1
5– 9	68	72	14	10
10–14	72	64	3	9
15–19	21	38	6	6
20–24	11	4	3	0
25–29	3	6	0	1
30–34	2	2	0	0
35–39	2	1	0	0
40–44	0	1	0	0
45–49	3	1	0	0
50+	3	4	0	0
Total	199	201	26	26

institution falls in the age group over 20 years.

The disease in this institution was clinically similar to that described as German measles in various textbooks. It was essentially afebrile, with occasional patients showing fever of 101 F or higher on the first day of illness. Constitutional symptoms appeared absent or minimal, and prodromata were not remarkable although evidence of mild coryza was present occasionally. There were no Koplik spots. The commonly described enlarged postcervical lymph nodes were observed in a large majority of the patients. The rash consisted of small light pink macules, usually discrete in nature, and occasionally scarlatiniform in appearance. Generally, the face and trunk were primarily involved, although the extremities likewise showed rash in many instances. This rash persisted for varying intervals from one to three days. In connection with establishing the diagnosis, frozen nasal washings and defibrinated blood were submitted to the National Institutes of Health for study. Some of this material has produced a German measles-like disease in monkeys.[10] Further studies are planned. One additional point bearing on the diagnosis is the absence of measles from the institution throughout this period. Measles was introduced during March, 1949, and during the ensuing two months 389 cases were reported, many of these illnesses occurring in those listed previously as cases of German measles.

10. Habel, K. Personal communication.

METHODS

Three separate studies were carried out using essentially the same technique but different lots of immune globulin.* The procedure was to determine the population exposed to German measles, to weed out those individuals who had already suffered from the disease during the current outbreak, to prepare an alphabetical list of the remaining individuals and then to immunize alternate persons on this list with 0.1 ml of immune globulin per pound of body weight. Past histories concerning infection with German measles were either lacking or completely unreliable, so that the true susceptibility of the population could not be accurately assessed. Following the immunization procedure, daily inspection of the children in the study was done by both nursing and medical personnel. Detailed clinical records were kept on the occurrence of illness. It is important to note that those participating in the follow-up studies had no definite knowledge as to which children had been immunized.

First study

The first trial of this prophylactic measure was initiated on August 5 and 6, when 199 inmates in two exposed buildings were immunized. These particular buildings housed male patients in the age groups illustrated in table 1. During the two-week period prior to initiating the study, 39 cases of German measles had been reported from these particular buildings, and it was assumed that the large bulk of the population had been effectively exposed. The dosage of immune globulin utilized differed slightly from that outlined above,

* The immune serum globulin (human) used in this study was furnished by the American Red Cross.

namely, patients weighing less than 50 pounds were given 5 ml, those weighing 50 to 99 pounds were given 10 ml, and those weighing over 100 pounds, 15 ml. Careful daily inspection of these patients during the ensuing three-week period failed to show any evidence of protection furnished by the immune globulin, as illustrated in tables 1 and 2. It will be noted that 26 cases of German measles occurred during this period both in the immunized and control groups. Review of the clinical records did not indicate any modification of the signs and symptoms of the disease produced by the immune globulin.

A question immediately arose concerning the quality of the immune globulin utilized. This particular lot (no. 8214-2) had been prepared over four years previously and had been considered outdated for three years. It had been utilized in this study since it represented the only material immediately available for this purpose at that time. Following the trial of this material in the prevention of German measles, immune globulin from the same lot was utilized in an attempt to prevent measles occurring in another State school. The material apparently prevented the occurrence of measles, although in view of the small sample of individuals studied, it was impossible accurately to estimate the degree of protection. Titration* of the H and O typhoid agglutinins, diphtheria antitoxin, and mouse protection against the P.R. 8 influenza virus failed to show any significant change in the level of these antibodies as compared to the determinations carried out four years previously on the same lot. Since the material had been adequately stored, it seems likely that the antibody content to most diseases had not deteriorated over this time interval. Nevertheless, in view of the remote possibility that this particular lot of immune globulin was at fault, it seemed essential to carry out further studies on this subject using fresh immune globulin. Accordingly, the epidemic at Rome was watched carefully so as to discover early the involvement of new buildings.

TABLE 2.—*German measles at Rome State School. Cases occurring in immunized and control groups by day of onset following administration of immune serum globulin, lot no. 8214-2. First study.*

	Days after administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Immunized	0	0	5	0	4	5	3	1	0	2	1	1	1	0	1	0	0	1	1	0
Control	0	2	1	1	3	4	2	2	0	1	3	2	0	0	0	0	0	2	3	0

Second study

On December 2, alternate children from three newly exposed wards (#33, #37, and #38) of X Building were immunized. This particular building housed on eight wards 322 female inmates, most of whom were children. In addition, a ward (R24) of adult female inmates, known to have been definitely exposed to the disease a few days prior, were also immunized in alternate alphabetical fashion. Immune globulin lot no. C3768 was utilized and the dosage was 0.1 ml per pound of body weight. Actually, in the adult ward consisting of 36 immunized and 36 control persons, no subsequent cases of German measles occurred. Likewise, in the infants' ward (#33), consisting of 17 immunized and 16 control patients, no cases developed during the four weeks

* These studies were performed through the kindness of Dr. Charles A. Janeway, Boston, Massachusetts.

TABLE 3.—*German measles at Rome State School. Age distribution of immunized and control groups by ward. Second and third studies.*

Age in years	Immunized by ward						Controls by ward						Grand total
	32	33	35	37	38	Total immunized	32	33	35	37	38	Total controls	
0–4	1	15	0	3	0	19	1	16	0	1	1	19	38
5–9	4	2	8	16	13	43	5	2	6	20	17	50	93
10–14	10	0	4	1	8	23	11	0	6	0	5	22	45
15–19	1	0	2	0	0	3	0	0	1	0	0	1	4
20+	1	0	0	0	0	1	0	0	0	0	0	0	1
Total	17	17	14	20	21	89	17	18	13	21	23	92	181

following immunization. Thus, the experience on these two wards did not serve to allow evaluation of the protection afforded by this procedure. However, in the other two wards (#37 and #38), definite evidence of protection was obtained. Detailed discussion of these findings will be presented in conjunction with the results of the third experiment.

Third study

On December 31, it became apparent that the infants' ward (#33) previously immunized had finally been re-exposed to German measles, three cases having just developed on the ward. In addition, two other wards (#32 and #35) had just been exposed in similar fashion. Accordingly, these three wards were immunized by the same alternating procedure outlined above, except that children on Ward #32 received twice the standard dose of immune globulin. The infants on Ward #33 immunized on this occasion were the same individuals immunized one month prior. A small portion of these children received the same lot of immune globulin used in the second study, but most of this group received a new lot (no. C3827). It soon became apparent that the new lot was giving very little protection. In table 3 the details concerning the number of persons on each ward studied, with their immunization status and age group, are presented. It will be seen that the age distribution of the immunized children was essentially identical with that of the control population.

The summarized results, in terms of the number of cases of German measles occurring in the remaining susceptible population during the interval three to 26 days after the date of immunization with each of the two lots of immune globulin, is presented in table 4. Since both lots were used on Ward #33 during the course of the third study, it seems legitimate to utilize the attack rates in the control population on this ward as a standard against which the attack rate in recipients of each lot can be compared. It will be noted that with the

TABLE 4.—*German measles at Rome State School. The occurrence of cases in immunized and control groups during the period 3 to 26 days after administration of immune serum globulin.*

Second study—globulin lot #C3768					Third study—globulin lot #C3827				
Ward	Number immunized	Cases	Number controls	Cases	Ward	Number immunized	Cases	Number controls	Cases
33	5	0	18	9	32	17	8	17	7
37	20	5	21	15	33	11	2	18	9
38	20	4	21	11	35	10	1	11	2
Total	45	9	60	35		38	11	46	18
Percent attack rate	20.0		58.4			29.0		39.1	

use of lot no. C3768, nine cases occurred among the 45 immunized children during the period three to 26 days after immunization, an attack rate of 20.0% as compared to 35 cases among the 60 remaining control individuals on these same wards, an attack rate of 55.5% for this same period. This difference is statistically very significant ($P = 0.0001$). In addition, based on the criimmunized children, an attack rate of 24.1% compared to 44 cases among 88 nonimmunized controls, or an attack rate of 50.0%. A difference as great or greater than this would be observed by chance alone only five times in 10,000 similar trials ($P = 0.0005$).

Actually, during the course of these experiments, 13 children who had been absent on the day of immunization were

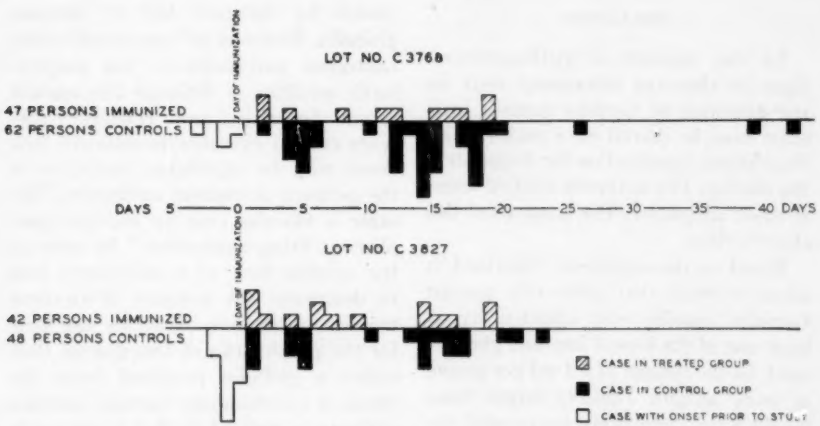
LOT NO. C 3768
DAY OF IMMUNIZATION
47 PERSONS IMMUNIZED
62 PERSONS CONTROLS
DAYS 5 0 5 10 15 20 25 30 35 40 DAYS
LOT NO. C 3827
DAY OF IMMUNIZATION
42 PERSONS IMMUNIZED
48 PERSONS CONTROLS
CASE IN TREATED GROUP
CASE IN CONTROL GROUP
CASE WITH ONSET PRIOR TO STUDY

FIG. 1.—German measles at Rome State School, 1948–49. Cases in treated and control groups by day of onset prior and subsequent to administration of two different lots of immune serum globulin.

teria of fever and extent and duration of the rash, the cases occurring among the immunized were in general somewhat milder than those in the control group.

On the other hand, the use of lot no. C3827 demonstrated a much less striking degree of protection, and the difference in attack rates in test and control groups (29.0% compared to 39.1%) was not statistically significant ($P = 0.3295$). Perhaps it is permissible, nevertheless, to combine the experience with these two lots of immune globulin, thus revealing during the period three to 26 days after giving immune globulin, 20 cases of German measles among 83 readmitted to the study wards. Ten of these later developed German measles during the study period. If one agrees that this group of readmissions may be considered legitimately as part of the control population, the calculated attack rates show a more striking contrast between immunized and control groups, and the added individuals tend to increase the statistical reliability of the results.

Figure 1 presents the experience graphically, indicating for each lot of immune globulin the day of onset of cases of German measles occurring in immunized and control populations. During the three months following the

completion of the study period (three to 26 days after immunization), only two additional cases of German measles occurred on the study wards. These, as indicated in figure 1, had onset on the 39th and 42nd days after immunization. These cases occurred among control children on Ward #38. Thus, the duration of effective passive immunity could not be properly assessed.

DISCUSSION

In the absence of pathognomonic signs or clear-cut laboratory tests for the diagnosis of German measles, reliance must be placed on a rather crude descriptive classification for diagnosis of the disease. The outbreak studied seems to meet adequately the demands of this classification.

Based on the experience described, it seems evident that protection against German measles was afforded by at least one of the lots of immune globulin used, in the dosage of 0.1 ml per pound of body weight. Possibly larger doses would have completely suppressed the infection. On the other hand, the other two lots of immune globulin failed to show significant evidence of protection within the limits set by this study. The life histories of these particular lots have been examined in some detail. Each met the standards of potency generally applied. Since the exact date of effective exposure to German measles is not known for any of the study population, and since the past history of the disease in this group could not be ascertained, further refinements in the analysis of the data are not possible with respect to the most opportune moment during the incubation period for passive immunization.

Careful study of the data presented would seem to indicate that the difference in the degree of protection shown in the three studies was a reflection of the presence or absence of adequate amounts of German measles antibody in the immune globulin. It seems likely, although this would be difficult to prove, that the explanation for this lies in a variation of the amounts of such antibodies in the donor population from whom the γ globulin was obtained. Reports have appeared on the variation in protective potency to other diseases shown by different lots of immune globulin. Stokes et al[11] apparently demonstrated variations in the prophylactic qualities of different lots against serum hepatitis, and Hartley[12] presents certain evidence to indicate that there may be significant variation in the potency of measles antibodies. The same is likewise true for mumps complement-fixing antibodies.[10] In view of the present lack of a laboratory test to determine the potency of German measles antibodies, it seems essential for the prophylaxis of this disease that either γ globulin prepared from the blood of convalescent German measles patients be utilized or that larger pools of adult blood be used in the production of immune globulin, so as to insure the inclusion of blood from persons carrying such antibodies. If several immunologically different strains of the German measles virus actually exist, this suggestion would be even more pertinent.

SUMMARY

1. Evidence has been presented demonstrating the prophylactic value of a particular lot of immune serum globulin in the prevention of German measles. During the period three to 26 days after receiving 0.1 ml per pound of body

11. Stokes, J., Blanchard, M., Neefe, J. R., Gellis, S. S. and Wade, G. R. 1948, J.A.M.A. **138**: 336.
12. Hartley, P. 1948. Lancet, **255**: 41.

weight of this lot, only nine cases occurred among 45 immunized persons (20.0%), as compared to 35 cases among 60 comparable controls (58.4%). The difference in attack rate is highly significant statistically.

2. One other lot of immune globulin failed to prevent the disease, while a third lot showed only slight protection. It seems likely that these conflicting results are a reflection of the relative potency of German measles antibodies in different lots of immune serum globulin. For the prevention of German measles in the exposed pregnant female, it seems essential that a known potent product be utilized, possibly immune serum globulin made from convalescent German measles serum. In the absence of such material there still may be merit to using the ordinary immune serum globulin for such protection.

THE EFFECT OF THIAMINE DEFICIENCY ON THE RESISTANCE OF RATS TO INFECTION WITH SPIROCHETES OF RELAPSING FEVER

K. GUGGENHEIM AND S. HALEVI

From the Laboratory of Nutrition, Hebrew University-Hadassah Medical School, Jerusalem, Israel

The effect of protein deficiency on the resistance of rats to infection with relapsing fever has been reported in a previous publication from this laboratory.[1] It was demonstrated that protein deficiency results in a more severe degree of infection, a longer duration of attacks and a high fatality rate. This diminished resistance was found to be due only to a minor degree to the concomitant caloric deficiency but to a greater extent to the protein deficiency per se. In order to elucidate further the role played by calories in specific nutritional deficiencies, thiamine deficiency was chosen. This paper presents results obtained by the infection with relapsing fever spirochetes of normal rats and of rats with various degrees of thiamine deficiency.

METHODS

The rats used were males which had been weaned one week.

The standard diet fed had the following composition: rice starch, 73; vitamin free casein, 18; olive oil, 5; and salt mixture, 4. One-tenth mg thiamine hydrochloride, 0.2 mg riboflavin, 5.0 mg nicotinic acid, 0.1 mg pyridoxine, 1.6 mg calcium pantothenate, 0.25 mg pteroylglutamic acid, and 100 mg choline chloride were incorporated per 100 g diet. In the thiamine-deficient diet thiamine was omitted, whereas in the thiamine subdeficient diet its amount was reduced to 0.02 mg per 100 g ration. Each rat received 100 I.U. vitamin A and 4 I.U. vitamin D twice weekly.

After two weeks on these diets the rats were infected intraperitoneally with *Borrelia persica*. The method of infection, the daily examination of the blood and the estimation of the degree of infection were the same as in our previous study.[1]

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RESULTS

Experiment 1.—Ninety-two rats were divided into three groups: group A, comprising 24 rats, received the standard diet ad libitum; group B, 34 rats, was fed the thiamine-deficient diet ad libitum; and group C, 34 rats, was given the standard diet, restricted in amount to the level of intake of the thiamine-deficient diet by group B. After two weeks on these diets 24 rats of each group were infected. The rats were observed only 12 days after infection (26 days on diet), because two-thirds of the infected rats of group B died in this period.

The food consumption and weight changes of these three groups are shown in table 1. As can be seen from the table, food consumption and weight increase in group A were normal, whereas the thiamine-deficient group B consumed markedly less food than the normal, control group. Accordingly, the growth performance of group B was very poor. Group C, pair-fed with group B but given the standard diet, showed a much smaller weight increase than group A.

As in our previous study no spirochetes were found in the blood of any rat during the first two days after infection. The incubation period ranged from two to five days, the mean periods being 3.6 days for group A, and 3.2 days for groups B and C. This difference was found to be statistically insignificant (table 2).

The periods during which spirochetes were present in the circulating blood

TABLE 1.—*Food consumption and weight changes of rats fed different diets.*

Exp. no.	Type of diet	Daily food consumption in g		Body weight in g	
		2 weeks before infection	During infection	At beginning of experiment	At day of infection
1	Group A: Standard (ad libitum)	9.5	11.5	39	82
	Group B: Thiamine deficient (ad libitum)	4.9	2.0	41	52
	Group C: Standard (pair-fed)	4.9	2.0	39	65
2	Group A: Standard (ad libitum)	9.3	11.3	39	79
	Group B: Thiamine subdeficient (ad libitum)	6.6	4.1	40	60
	Group C: Standard (pair-fed)	6.6	4.1	39	68

varied between one and seven days with an average of 1.8 days in group A, 3.6 days in group B, and 2.8 days in group C. The mean differences observed between groups A and B and those between A and C proved to be highly significant statistically.

Considerable differences were observed in the relative degree of infection, as can be seen from table 3. The mean degree of infection was found to be more severe in group B than in group A. It appears that the diminished resistance of the thiamine-deficient group was due only partially to a specific thiamine deficiency but to a greater extent to the reduced food intake. This was indicated by the results obtained with group C, which was given the standard diet (as group A) but in restricted amounts (as was eaten by group B). The response of this group was more like that of group B than group A. The probability that the mean difference between B and C was due to chance was found to be 1/100, whereas that between A and B, and between A and C even less than 1/1000.

The infection with *B. persica*, though not fatal in rats fed the standard diet, resulted in a high mortality rate among the thiamine-deficient rats. Table 4 shows that 16 out of 24 infected rats kept on the thiamine-deficient diet died in the course of the experiment, whereas in the uninfected, control group of ten

TABLE 2.—*Incubation time and duration of attacks in rats infected with* B. persica *and fed different diets.*

Exp. no.	Dietetic group	Incubation time in days			Duration of attacks in days		
		Mean	Standard deviation	Standard error	Mean	Standard deviation	Standard error
1	A	3.6	1.55	0.32	1.8	0.77	0.12
	B	3.2	0.66	0.13	3.6	2.05	0.32
	C	3.2	0.45	0.10	2.8	1.93	0.31
	P* = A/B			0.3			0.01
	A/C			0.2			0.01
	B/C			1.0			0.1
2	A	2.9	0.70	0.14	2.0	1.19	0.14
	B	2.9	0.87	0.18	1.9	1.52	0.18
	C	2.8	0.70	0.14	2.0	1.09	0.13
	P* = A/B			1.0			0.7
	A/C			0.6			1.0
	B/C			0.7			0.7

* Probability that the mean differences observed are due to chance.

TABLE 3.—*Degree of infection with* B. persica *in rats fed different diets.*

Day of infection	Means and standard deviations					
	Experiment 1			Experiment 2		
	A	B	C	A	B	C
3	0.2 (0.77)	0.2 (0.57)	0	0.6 (0.87)	0.4 (0.62)	0.5 (0.66)
4	1.4 (1.38)	1.8 (1.90)	1.6 (0.96)	2.8 (2.21)	2.9 (2.00)	2.9 (1.89)
5	2.2 (1.89)	3.0 (1.97)	3.1 (1.67)	3.6 (2.50)	2.9 (1.65)	3.4 (2.04)
6	1.0 (1.49)	2.4 (1.39)	3.4 (2.26)	2.1 (3.32)	1.5 (1.91)	2.3 (3.09)
7	0	2.9 (1.93)	1.7 (1.98)	0.3 (0.47)	0.6 (0.94)	0.5 (1.06)
8	0.2 (0.61)	2.3 (2.79)	1.3 (1.41)	0.3 (0.77)	0.1 (0.34)	0.1 (0.28)
9	0.4 (1.01)	2.3 (2.95)	1.0 (1.70)	0.7 (1.22)	0.9 (1.33)	0.4 (0.57)
10	0.9 (1.33)	2.0 (2.74)	0.9 (1.32)	0.8 (1.05)	0.3 (0.79)	1.0 (1.48)
11	0.8 (1.15)	2.0 (2.10)	1.0 (1.24)	0.2 (0.44)	0.2 (0.42)	0.8 (1.33)
12	0.4 (0.74)	1.1 (1.24)	0.7 (1.05)	0.2 (0.82)	0.6 (1.02)	0.4 (1.54)
13				0.3 (0.66)	0.2 (0.69)	0.7 (1.47)
14				0.5 (1.25)	0.3 (0.89)	0.2 (0.49)
15				0.2 (0.66)	0.3 (1.14)	0
16				0.7 (1.44)	0.3 (1.14)	0.5 (0.94)
17				0.6 (0.97)	0	0.7 (1.27)
18				0.5 (1.16)	0.3 (0.91)	0.7 (1.53)
19				0.9 (1.56)	0.6 (1.47)	0.4 (0.90)
20				0.5 (1.38)	0.3 (0.71)	0.4 (1.08)
21				0.6 (1.33)	0.7 (1.47)	0.6 (1.54)
Mean	0.7 (1.31)	2.0 (2.16)	1.5 (1.76)	0.9 (1.70)	0.7 (1.82)	0.9 (1.69)
Standard error	0.08	0.14	0.13	0.08	0.09	0.08
P	A/B	0.001		A/B	0.1	
	A/C	0.001		A/C	1.0	
	B/C	0.01		B/C	0.1	

rats, only two died. In the pair-fed infected group, nine out of 24 rats died as compared with four out of ten rats in the noninfected pair-fed group. These figures and their statistical analysis demonstrate also the relative similarity of response of groups B and C as contrasted to the highly significant differences in mortality between group A (infected) and groups B and C (infected). It may be concluded, therefore, that the diminished resistance of the deficient rats to spirochetal infection is due partially to an inanition effect. To elucidate this point further experiment 2 was performed.

TABLE 4.—*Mortality of noninfected rats and of rats infected with* B. persica *fed different diets.*

Exp. no.	Dietetic group	Infected	Number of rats	Number of rats that died during experiment	Average number of days before death of rats that died
1	A	+	24	0	
	B	+	24	16 (66%)	25.8
	B	−	10	2 (20%)	26.0
	C	+	24	9 (36%)	24.7
	C	−	10	4 (40%)	25.2
2	A	+	24	0	
	B	+	24	3 (12%)	26.3
	B	−	12	0	
	C	+	24	2 (8%)	30.0
	C	−	12	0	

Dietetic groups compared	Exp. 1	Exp. 2
	P value	*P* value
A infected/B infected	0.001	0.20
A infected/C infected	0.001	0.50
B infected/B not infected	0.05	0.50
B infected/C infected	0.05	0.95
B not infected/C not infected	0.50	1.00
C infected/C not infected	0.95	0.40

Experiment 2.—In this experiment group B received a thiamine-subdeficient diet, i.e., a diet which contained thiamine but in restricted amount—20% of the quantity supplied with the standard diet. A further difference between the two experiments lay in the observation period during the infection; the low mortality of the deficient group of experiment 2 allowed its extension to three weeks. In all other respects the set-up of this experiment was identical with that of experiment 1. The results obtained are also given in tables 1 through 4.

The food consumption of the thiamine-subdeficient group B was considerably higher than that of the rats deprived entirely of this vitamin during the two weeks preceding infection, and even higher during the infection period.

As a result growth was not so depressed as in the comparable thiamine-deficient group of the first experiment. The response to infection with spirochetes was found to be similar in all of the three groups. No statistically significant difference could be revealed in incubation time, duration of attacks, mean degree of infection, or mortality. In spite of the markedly diminished food intake and the resultant depression of growth induced by this mild form of thiamine deficiency, the resistance of the organism to infection with spirochetes was found to be unimpaired. The restriction of food intake, however, was not severe enough to increase the susceptibility to infection. Thus, the results of this experiment also support the view that the lowered resistance to spirochetal infection in thiamine deficiency is to a great extent the result of the reduced food intake and the concomitant caloric deficiency.

DISCUSSION

Our experiments show that thiamine deficiency in rats leads to a lowered resistance to infection with spirochetes of relapsing fever. Two factors play a role in this situation: the lack of the specific nutrient, thiamine, and the concomitant restriction of caloric intake. With a mild degree of thiamine deficiency, which is accompanied by a 30% reduction of food intake during the two weeks before infection, no impairment of resistance to infection could be found. Moreover, the deficiency of thiamine was not severe enough to decrease the resistance. When food lacks thiamine completely and the animals consume voluntarily 50% less food than normal controls, a point is reached where the adaptation to the diminished food intake fails, and the susceptibility increases. In this severe degree of thiamine deficiency the lack of the specific nutrient plays an additional role. The thiamine-deficient rats showed a more severe degree of infection and much higher mortality rate than the pair-fed controls. These differences were found to be statistically significant.

In our previous study on protein deficiency[1] we demonstrated that the diminished resistance of protein-deficient rats was mainly the result of the specific lack of protein and only to a minor degree the effect of the concomitant caloric deficiency. This result can be explained by the specific pattern of food intake and the resultant growth in these two forms of nutritional deficiency Details are shown in table 5. Normal young rats ate about 9.5 g food per day in the preinfection period, and their weight increased in this time by 43 g. The food intake rose an additional 21% during the infection period. Comparable protein deficient rats and also their pair-fed controls ate only 3.9 g per day before the infection. With this food intake their weight decreased considerably even before infection, whereas that of the pair-fed controls remained more or less constant. Thiamine-deficient rats and their pair-fed controls

TABLE 5.—*Food intake and weight changes of rats maintained on diets deficient in protein or in thiamine.*

Diet	Daily food consumption in g			Weight changes in g
	During the first two weeks experiment *a*	During infection *b*	*b* as percent of *a*	during the first two weeks of experiment
Standard (ad libitum)	9.5	11.5	121	+43
Protein deficient	3.9	2.5	64	− 7
Pair-fed	3.9	2.5	64	+ 5
Thamine deficient	4.9	2.0	41	+ 9
Pair-fed	4.9	2.0	41	+26

1. Guggenheim, K., Buechler-Czaczkes, E. and Halevi, S. 1951, J. Infect. Dis. 88: 105–108.

consumed more food during the same period—4.9 g per day. This intake resulted in a marked weight increase, 9 and 26 g, respectively. The further development of the deficiency showed a characteristic trait: in the protein deficient rats a slow decrease of voluntary food intake, and in the thiamine deficient group a sudden and severe one. The gradually restricted supply did not greatly harm the pair-fed rats of the protein-deficient group. None of the noninfected rats died during the experiment and their resistance to infection was not greatly impaired. On the contrary, the pair-fed rats of the thiamine-deficient group, which had gained 26 g in the first two weeks, were forced to cut down their food intake drastically (by 59%). This sudden reduction led to a high mortality rate of about 40% of both the infected and uninfected groups, and induced a breakdown of the resistance to infection. A similar deleterious effect on the resistance to infection brought about by sudden restriction of the caloric supply has been demonstrated by one of us[2,3] studying the effect of nutritional deficiency on the course of salmonella infection in rats.

An outstanding result of various animal experiments is the demonstration that undernutrition has different effects with different agents and different animal species. Decreased resistance or increased susceptibility has often been reported and claimed in undernourished or malnourished animals. Inanition has been reported to decrease resistance (chicks, *Plasmodium lophurae*),[4] to be without effect (rats, pneumococci),[5] and to increase resistance (mice, poliomyelitis).[6]

As to the effect of thiamine, Badger et al[7] observed a diminished resistance to rat leprosy in thiamine deficiency. A high susceptibility to experimental pneumococcal infection was shown by Wooley and Sebrell[8] in mice and by Robinson and Siegel[5] in rats. The diminished resistance of mice was found to be due to specific avitaminosis, the concomitant inanition having no apparent influence. Thiamine deficiency lowers the resistance of rats to infection with *Nippostrongylus muris*[9] but does not influence in any recognizable manner the susceptibility to an intravenous injection of avian tubercle bacilli.[10] Rats and mice kept on a thiamine-deficient diet showed diminished resistance to an infection with *Salmonella typhimurium*.[11] In rats the heightened susceptibility is secondary to inanition, whereas in mice it is a primary result of the thiamine deficiency. The claim of Seeler and Ott[12] that thiamine deficiency decreases the resistance of chicks to an infection with *Plasmodium lophurae* was based on small differences. Roos et al,[13] working with chicks and ducks, found no decrease in resistance. On the other hand, thiamine-deficient mice exhibit an increased resistance to infection with Theiler virus, poliomyelitis virus and the virus of western equine en-

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cephalitis.[6,14–18] However, Clark et al[19] showed in studies with *Macaca mulatta* that this species, when deficient in thiamine, does not exhibit increased resistance to poliomyelitis virus.

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SUMMARY

1. Young rats maintained during two weeks on a thiamine-deficient diet were inoculated intraperitoneally with *Borrelia persica*. They displayed a more severe degree of infection, a longer duration of attacks and a higher fatality rate than control rats given a full diet ad libitum.

2. The diminished resistance of thiamine-deficient rats was only to a minor degree the result of the thiamine deficiency per se but to a larger extent the effect of the concomitant caloric deficiency.

3. Rats fed a thiamine-subdeficient diet did not show any impairment in their resistance to an infection with relapsing fever spirochetes.

ANTIMETABOLIC ACTION OF SULFADIAZINE AND CERTAIN ANTIBIOTICS FOR BRUCELLA

NORMAN B. MCCULLOUGH* AND GRACE A. BEAL*

Among the agents currently employed in the treatment of brucellosis are sulfadiazine, streptomycin or dihydrostreptomycin, aureomycin, chloromycetin, and, most recently, terramycin. The in vitro effectiveness of these compounds in inhibiting the growth of brucella has been established (Wise, 1942; Worley et al, 1944; Smith et al, 1948; Yow and Spink, 1949; McLean et al, 1949; Herrell et al, 1950). The effect of combinations of certain of these drugs has been cited both in the treatment of human brucellosis and in experimental brucellosis of laboratory animals. Synergistic activity of a combination of streptomycin and sulfadiazine has been reported by Pulaski and Amspacher (1947), Eisele and McCullough (1947), and Spink et al (1948) in the treatment of human brucellosis, and by Shaffer and Spink (1948) and Carle and Larson (1950) in experimental brucellosis of laboratory animals. The value of a combination of dihydrostreptomycin and aureomycin has been reported by Herrell and Barber (1949 and 1950) in the treatment of human brucellosis, and by Magoffin et al (1949), Heilman (1949), Carle and Larson (1950), and Werner and Knight (1950) in experimentally infected laboratory animals. Eisele and McCullough (1949, 1950a and b) have used a combination of dihydrostreptomycin, aureomycin, and sulfadiazine successfully in the treatment of human brucellosis.

The present study is concerned primarily with the effect of aureomycin, streptomycin and dihydrostreptomycin, and sulfadiazine on oxygen uptake of washed cell suspensions of brucella. Limited observations have been made on the influence of chloromycetin and terramycin on the respiration of brucella.

MATERIALS AND METHODS

Bacterial cultures.—The strains of *Brucella melitensis*, *Brucella suis*, and *Brucella abortus* had been recently isolated in the authors' laboratory from human cases of brucellosis. The characteristics of all strains (carbon dioxide requirement, hydrogen sulfide production, growth on dye mediums, and serological reactions) were typical of the respective species; all produced S type colonies and were pathogenic for guinea pigs.

Drugs.—The following compounds were used: streptomycin hydrochloride (Merck, lot no. 535); dihydrostreptomycin sulfate (Heyden, lot no. HS 207); chloromycetin (Parke, Davis and Company, RX2969H); aureomycin hydrochloride (Lederle); terramycin hydrochloride (Chas. Pfizer and Company, lot no. WLP 507022); sodium sulfadiazine (Lederle).

Manometric technique.—The brucella cultures were grown for 24 to 36 hours on beef liver infusion agar at 37 C. The cells were harvested in 30 ml volumes of sterile 0.05 *M* phosphate buffer, pH 6.8, filtered, and washed three times by centrifuging and resuspending in fresh buffer solution. The cell suspensions were diluted to a standard turbidity (Klett-Summerson photoelectric colorimeter) which approximated 20 to 30 billion viable cells per ml, and stored in the refrigerator at 4 to 6 C prior to use.

Oxygen uptake was determined at 37 C in a normal atmosphere by the conventional Warburg technique (Umbreit et al, 1949) using 15 ml flasks with a single side arm. The final fluid volume in all flasks was 2.0 ml, consisting of 1 ml of cell suspension, 0.5 ml of 0.05*M* phosphate buffer, pH 6.8, and 0.5 ml total volume of other reagents (carbohydrate and/or drugs). The cen-

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From the Department of Medicine, The University of Chicago, Chicago 37, Illinois, and the Federal Security Agency, Public Health Service, National Institutes of Health, Bethesda, Maryland.

* Present address: Federal Security Agency, Public Health Service, National Institutes of Health, Microbiological Institute, Bethesda, Md.

ter well received 0.2 ml of 20% potassium hydroxide. The carbohydrates previously sterilized by sintered glass filtration were used in a final concentration of 0.5%. Solutions of the antibiotics and sulfadiazine were freshly prepared in sterile redistilled water for each experiment. After placing the manometers in the water bath, a minimal 20 minute equilibration period was allowed before beginning measurement of oxygen uptake. Measurements were then made over a 15 to 30 minute period to assure an approximately equal respiratory status in the duplicate flasks before addition of the critical components of the various systems. The rate of shaking was adjusted to 120 oscillations per minute. At the conclusion of each test run, brom thymol blue indicator was added to the flasks to assure that the systems were adequately buffered and that the potassium hydroxide had not spilled over during the run.

The manometric measurements recorded for each experiment, unless otherwise stated, were those actually determined for the various systems under study without subtraction of the endogenous respiration values which are listed separately. Calculations of percent change in oxygen consumption were likewise made by direct comparison of the reading obtained in the presence of a particular drug with the uptake measured in the corresponding drug-free control without subtracting the endogenous respiration values from either.

TABLE 1.—*The effect of aureomycin on oxygen uptake of brucella.*

Systems	*Br. abortus*		*Br. melitensis*		*Br. suis*	
	Microliters O_2 uptake	Percent decrease	Microliters O_2 uptake	Percent decrease	Microliters O_2 uptake	Percent decrease
1. Endogeneous respiration	38		47		39	
2. Endogenous + 500 μg aureomycin (initially present)	23	39.5	21	55.3	23	41.0
3. Glucose control	150		176		496	
4. Glucose + 500 μg aureomycin (simultaneously added)	44	70.7	45	74.4	116	76.6
5. Glucose + 500 μg aureomycin (initially present)	38	74.7	27	84.7	66	86.7
6. Glucose + 200 μg aureomycin (initially present)	90	40.0	41	76.7	271	45.4

Materials added from side arm: #1, #2 (water); #3 (glucose); #4 (glucose and aureomycin); #5, #6 (glucose). The period of observation for *Br. abortus* was 4.5 hr., for *Br. melitensis* 5 hr., for *Br. suis* 5.5 hr.

EXPERIMENTAL RESULTS

The effect of aureomycin on oyxgen uptake of Br. abortus, Br. melitensis *and* Br. suis.—The effect of aureomycin on endogenous respiration and on oxygen uptake with glucose substrate was determined for *Br. abortus*, *Br. melitensis*, and *Br. suis*. Results typical of those obtained in several experiments are summarized in table 1. The data, combined for convenience, represent readings obtained in three runs using three separate cell suspensions. Values listed for each species represent concurrent determinations using equal volumes of the same homogeneous cell suspensions. The cell suspensions were allowed to respire at 37 C for 60 minutes before the side arm contents were added. In the experiments where aureomycin and glucose were added simultaneously, these substances were both placed in the side arm and added at the end of the endogenous control respiration period. Where aureomycin is designated as initially present, it was added to the main compartment of the Warburg vessels at the time the cells were added. In the concentrations used (200 to 500 μg per Warburg flask), aureomycin interfered with oxygen uptake for all three species of brucella in both the absence and presence of glucose. In addition to the test series listed in table 1, experiments were performed which demonstrated that the addition of aureomycin to a system in which glucose oxidation was actively proceeding produced a prompt and continuous decrease in rate of oxygen uptake with all three species.

The effect of aureomycin on oxygen up-

TABLE 2.—*Effect of aureomycin, chloromycetin, streptomycin, and sulfadiazine on oxygen uptake of* Br. melitensis *in the presence of glucose over a five-hour period.*

Systems	Microliters O_2 uptake	Percent difference*
1. Endogenous respiration	46	
2. Glucose control	254	
3. Glucose+200 μg aureomycin	127	−50.0
4. Glucose+100 μg aureomycin	163	−35.8
5. Glucose+400 μg chloromycetin	214	−15.7
6. Glucose+200 μg chloromycetin	224	−11.8
7. Glucose+1000 μg streptomycin	264	+ 3.9
8. Glucose+10 mg sulfadiazine	259	+ 2.0

Conditions: The drugs were added from the side arm 30 minutes after measurement of oxygen uptake of cells with glucose substrate had assured equal rates for all flasks. The sum uptake recorded does not include this preliminary measurement.
* Calculated from glucose control oxygen uptake.

take of Br. melitensis *with pyruvate substrate.*—For a four-hour period of observation, the oxygen uptake of cells in the presence of 0.5% sodium pyruvate was 274 μl. The uptake of cells to which pyruvate and aureomycin (400 μg) were added simultaneously was 98 μl, an inhibition of 64.2%. Endogenous respiration for the period was 84 μl.

The effect of aureomycin on oxygen uptake of Br. suis *with D-trehalose, D-fructose, and D-xylose substrates.*—Aureomycin (400 μg) and the individual carbohydrates were added simultaneously from the side arm; control flasks contained the same concentration of carbohydrates and the appropriate volume of water instead of aureomycin. After addition of the antibiotic and carbohydrate substrate, readings were continued for two hours. The microliters of oxygen consumed and percent decrease in the presence of aureomycin were: D-fructose, 135 (53.9); D-xylose, 119 (32.9); D-trehalose, 71 (52.0). Endogenous respiration for the period was 19 μl.

Comparison of the effect of aureomycin on oxygen uptake of an aerobic and of a carbon dioxide-sensitive strain of Br. abortus *with glucose substrate.*—The aerobic strain was grown in a normal atmosphere and the carbon dioxide-sensitive parent strain in the presence of 10% added carbon dioxide. In all other respects the cells were treated similarly. Glucose and aureomycin (200 μg) were added simultaneously to the cell suspensions and manometric readings were made over a five-hour period. The reduction in oxygen uptake in the presence of aureomycin for the aerobic and carbon dioxide-sensitive strains was the same, being 44.3 and 44.0%, respectively.

Continued effect of aureomycin on Br. melitensis *cells after incubation and washing.*—A homogeneous cell suspension was divided into two equal volumes. To one portion was added aureomycin in a final concentration of 200 μg/ml. The other sample received an equivalent volume of distilled water. The cell suspensions were incubated at 37 C for 30 minutes, washed three times with water, and resuspended and adjusted to the original density in buffer. Over a three-hour period of observation, the oxygen uptake with glucose substrate of cells incubated in the presence of aureomycin was 82 μl, as compared with the control uptake of 203 μl, an inhibition of 59.6%. Endogenous respiration was 62 μl.

The effect of growth factors on aureomycin inhibition of respiration of Br. melitensis.—Established growth factors for brucella (thiamin, nicotinic acid, and biotin), in concentrations 100 times those advocated for growth in a synthetic medium, were not effective in modifying aureomycin (100 μg/ml) inhibition of oxygen uptake of cells in the presence or absence of glucose. (Oxygen consumption was measured over a period of four hours.) This concentration of growth factors likewise had no apparent influence on endogenous respiration or oxygen uptake with glucose substrate in aureomycin-free systems.

The effect of chloromycetin, streptomycin, and sulfadiazine on oxygen uptake of Br. melitensis *with glucose substrate.*—Aureomycin was included for comparison and its effect was again demonstrated, both in the concentration previously shown to be inhibitory and in a concentration one half this strength (100 μg/flask). The results are presented in table 2. Greatest decrease in oxygen uptake occurred with aureomycin. Chloromycetin, in a concentration of 200 to 400 μg per flask, produced a significant reduction in oxygen uptake, but this was not as great as that caused by equal and lesser amounts of aureomycin. The total microliters of oxygen consumed in the presence of 1000 μg of streptomycin was in slight excess of that of the control. However, interval readings revealed that the rate of oxygen uptake during the final hour was 6% less than that for the control. No inhibition was demonstrated with sulfadiazine, either in interval or sum readings.

The effect of terramycin on oxygen uptake of Br. melitensis *with glucose substrate.*—Over a four-hour period, the oxygen uptake of cells in the presence of terramycin (400 μg added simultaneously with glucose) was 39.6% of that of the control. Other tests were conducted in which the terramycin was added after cells had been in contact with glucose for 30 minutes before the terramycin was introduced; the addition of terramycin resulted in a decrease in rate of oxygen uptake.

TABLE 3.—*The combined effect of aureomycin and dihydrostreptomycin on oxygen uptake of* B. melitensis *with glucose substrate.*

Systems	Microliters O_2 uptake	Percent decrease
A. Period of observation: 3 hours		
1. Endogenous respiration	62	
2. Glucose control	203	
3. Glucose + 1000 μg dihydrostreptomycin	201	1.0
4. Glucose + 100 μg aureomycin	72	64.5
5. Glucose + 100 μg aureomycin + 1000 μg dihydrostreptomycin	79	61.1
B. Period of observation: 4 hours		
1. Endogenous respiration	74	
2. Glucose control	297	
3. Glucose + 50 μg aureomycin	132	55.6
4. Glucose + 20 μg aureomycin	203	31.6
5. Glucose + 1000 μg dihydrostreptomycin	287	3.4
6. Glucose + 20 μg aureomycin + 1000 μg dihydrostreptomycin	118	60.3
C. Period of observation: 5 hours		
1. Endogenous respiration	144	
2. Glucose control	569	
3. Glucose + 20 μg aureomycin	357	37.3
4. Glucose + 20 μg aureomycin + 1000 μg dihydrostreptomycin	185	67.5
5. Glucose + 10 μg aureomycin	521	8.4
6. Glucose + 10 μg aureomycin + 1000 μg dihydrostreptomycin	294	48.3

Conditions: drugs present initially, glucose added to systems after 30 minutes at 37 C.

The combined effect of aureomycin and dihydrostreptomycin on oxygen uptake of Br. melitensis *with glucose substrate.*—Table 3 summarizes the results of typical experiments illustrating the combined effect of aureomycin and dihydrostreptomycin on oxygen uptake of *Br. melitensis* with glucose substrate. Data listed in section A, table 3, again show, as was demonstrated earlier, definite reduction of oxygen uptake in the presence of 100 μg aureomycin, and none during the time observed for 1000 μg dihydrostreptomycin. When the two were combined in the same concentrations as used singly, there was no evidence of inhibition other than that caused by aureomycin alone. By decreasing the amount of aureomycin to 20 μg and leaving the dihydrostreptomycin in a concentration of 1000 μg per flask, there was demonstrated a definite

synergistic effect (section B, table 3). When added singly, the total percent reduction in oxygen uptake was 35.0 (3.4% by dihydrostreptomycin and 31.6% by aureomycin). When combined in the same concentration as used singly, the percent decrease in oxygen uptake was 60.3. These points have been graphically illustrated in figure 1. drug singly. Usually, the amount of oxygen consumed in the presence of sulfadiazine was slightly greater than that of the control. Unlike streptomycin and dihydrostreptomycin, this apparent stimulation in oxygen uptake by sulfadiazine was continuous. In cases where readings were made at intervals over several hours, there was no indication

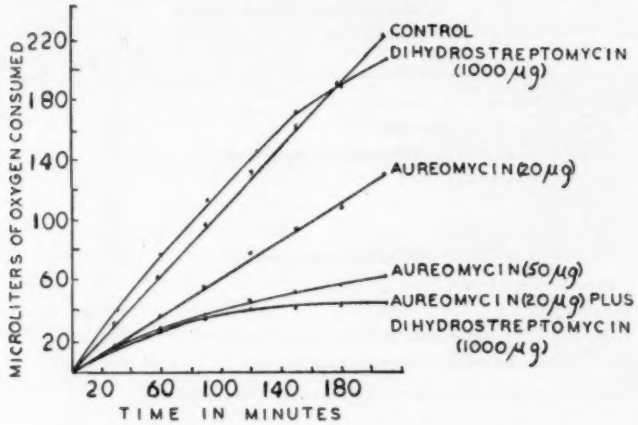

FIG. 1.—The combined effect of dihydrostreptomycin and aureomycin on oxygen uptake of *Br. melitensis* with glucose substrate (endogenous values subtracted).

In section C, table 3, the concentration of aureomycin was reduced to 10 μg and produced measurable inhibition; when the same concentration of aureomycin was combined with 1000 μg of dihydrostreptomycin, there was a 39.9% further reduction in oxygen uptake.

The effect of sulfadiazine singly and in combination with aureomycin and/or dihydrostreptomycin.—A series of experiments were performed in an attempt to demonstrate that sulfadiazine affected respiration of nonproliferating cells of *Br. melitensis*. The concentration range employed varied from 10 to 500 mg%. The results of certain of these studies are listed in table 4. In no instance was there noted any reduction in oxygen uptake in the presence of this of eventual reduction in rate of oxygen uptake in the presence of this drug. Furthermore, the addition of sulfadiazine to inhibitory concentrations of aureomycin (10 to 20 μg) lessened the effect of the latter compound. The effectiveness of combinations of dihydrostreptomycin and aureomycin in reducing oxygen uptake was also lessened when sulfadiazine was present.

Although inhibitory action by sulfadiazine for brucella was not demonstrated manometrically using washed cell suspensions, the inhibition of oxygen uptake of growing cultures of *Br. melitensis* by sulfonamide compounds has been reported (Kempner et al, 1940). In studies conducted in this laboratory (McCullough and Beal, 1948), the in-

hibition of growth of brucella by sulfadiazine, as reported by other workers, was observed, and, in addition, evidence was obtained which indicated a synergistic effect on growth inhibition when sulfadiazine was combined with streptomycin. A strain of *Br. abortus* was inoculated into flasks of trypticase soy broth containing the two drugs singly and in various combinations. Plate counts were made immediately which established that an approximately equal number of viable cells was initially present in each flask. The flasks were then incubated at 37 C in the presence of 10% added carbon dioxide. Viable cell counts were made of aliquots from each flask at the 12th and 24th hour of incubation and at periodic intervals thereafter over a 14-day period. Dilutions for plate counts were made in saline containing p-aminobenzoic acid (1 mg%) and plated to the surface of trypticase soy agar, modified by the addition of p-aminobenzoic acid. Sulfadiazine in a final concentration of 10 mg% was inhibitory to the extent that turbidity did not develop from the initial inoculum of 5000 cells/ml. Plate counts revealed an increase in numbers of cells (the equivalent of seven cell divisions) through the 48th hour of incubation, followed by a gradual decline. Viable organisms were recovered up to and including the tenth but not on subsequent days. Flasks containing 2 μg/ml of streptomycin did not develop turbidity until the 96th hour of incubation. Plate counts revealed an initial increase in cell numbers during the first 12 hours (the equivalent of three cell divisions), then a gradual decline in cell count, which at the 48th hour was less than that originally inoculated. During the ensuing 24 hours there was a rapid increase in cells, continuing until a number equal to that in the control flasks was attained. The combination of 2 μg/ml of streptomycin with 10 mg% sulfadiazine did not allow sufficient growth for turbidity to develop. Plate counts revealed a slight increase during the first 24 hours of incubation (the equivalent of two cell divisions), fol-

TABLE 4.—*Effect of sulfadiazine, singly and in combination with aureomycin and/or dihydrostreptomycin, on oxygen uptake of* B. melitensis *in the presence of glucose.*

Systems	Microliters O_2 uptake	Percent difference
A. Period of observation: 2.5 hours		
1. Endogenous respiration	37	
2. Glucose control	133	
3. Glucose + 1 mg sulfadiazine	146	+ 9.8
4. Glucose + 1 mg sulfadiazine + 1000 μg dihydrostreptomycin	132	− 0.8
5. Glucose + 1 mg sulfadiazine + 100 μg aureomycin	30	−77.4
6. Glucose + 100 μg aureomycin	33	−75.2
7. Glucose + 100 μg aureomycin + 1000 μg dihydrostreptomycin	30	−77.4
8. Glucose + 1 mg sulfadiazine + 100 μg aureomycin + 1000 μg dihydrostreptomycin	29	−78.2
B. Period of observation: 5 hours		
1. Endogenous respiration	144	
2. Glucose control	569	
3. Glucose + 20 μg aureomycin	357	−37.3
4. Glucose + 1 mg sulfadiazine + 20 μg aureomycin	486	−14.6
5. Glucose + 20 μg aureomycin + 1000 μg dihydrostreptomycin	185	−67.5
6. Glucose + 1 mg sulfadiazine + 20 μg aureomycin + 1000 μg dihydrostreptomycin	237	−58.3
C. Period of observation: 4.5 hours		
1. Endogenous respiration	196	
2. Glucose control	677	
3. Glucose + 0.2 mg sulfadiazine	691	+ 2.1
4. Glucose + 10 μg aureomycin	617	− 8.8
5. Glucose + 0.2 mg sulfadiazine + 10 μg aureomycin	681	+ 0.6
6. Glucose + 10 μg aureomycin + 1000 μg dihydrostreptomycin	420	−38.0
7. Glucose + 0.2 mg sulfadiazine + 10 μg aureomycin + 1000 μg dihydrostreptomycin	479	−29.c

Conditions: drugs present initially, glucose added from vessel side arm after 30 minutes at 37 C.

lowed by a sharp drop to a number 1/50th of that initially present by the 48th hour; by the 72nd hour, the solution was completely sterilized. Flasks containing 1 μg/ml of streptomycin remained clear until the 48th hour, but there was never a decrease in viable cell numbers, and by the 6th day, maximal growth equal to that of the control was attained. With 1 μg of streptomycin combined with 10 mg% of sulfadiazine visible turbidity did not develop. Plate counts revealed no increase in cell numbers after the 24th hour and then a consistent decline with sterility resulting by the 120th hour. Flasks containing 0.5 μg/ml of streptomycin maintained growth equal to that of the control throughout each interval. When combined with sulfadiazine there was an increase in cell numbers until the 24th hour (the equivalent of 5 cell divisions), followed by a gradual decline. There was complete sterilization by the seventh day.

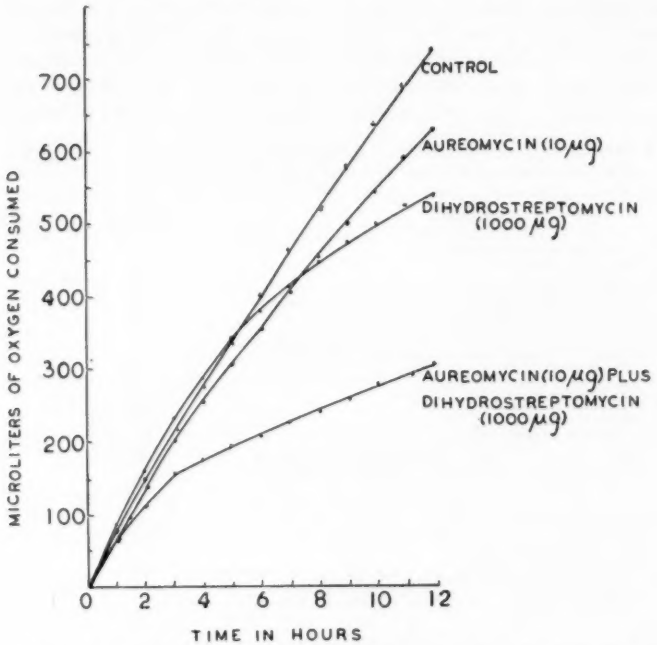

FIG. 2.—Influence of time on the effect of oxygen uptake of *Br. militensis* by aureomycin, dihydrostreptomycin, and aureomycin combined with dihydrostreptomycin. Glucose added at 0 hour. (Antibiotics in presence of cells one hour at room temperature and 35 minutes at 37 C before addition of glucose; endogenous respiration values subtracted.)

Influence of length of time of exposure to drugs on oxygen uptake of Br. melitensis *with glucose substrate.*—The action of drugs on oxygen uptake was found to be considerably influenced by the length of time the cells and drugs were together. This was well demonstrated in an experiment in which manometric measurements were made at 30-minute

intervals over a 12-hour observation period. The systems under observation included 10 μg aureomycin, 1000 μg dihydrostreptomycin, and 10 μg aureomycin combined with 1000 μg dihydrostreptomycin. The drugs were placed in the flasks at room temperature immediately prior to the addition of cells. Control flasks received the appropriate volume of water instead of drug. An equal amount of glucose was placed in the side arm of each flask. The cells were in contact with the drugs for one hour at room temperature and 35 minutes at 37 C before the glucose was tipped in. The over-all effect of the drugs on oxygen uptake for the 12-hour period is graphically illustrated in figure 2. The percent decrease in total oxygen uptake of the various systems in relation to the control were: 10 μg aureomycin (14.0), 1000 μg dihydrostreptomycin (26.7), 10 μg aureomycin combined with 1000 μg dihydrostreptomycin (57.8).

A better insight into the effect of the drugs singly and in combination, however, is gained from interval observations and calculations of percent change in oxygen uptake per hour. Table 5, covering the first six hours, presents the significant data.

TABLE 5.—*Relationship of time to effect of aureomycin and dihydrostreptomycin singly and in combination on oxygen uptake of* Br. melitensis.

Interval	Microliters O_2 uptake	Percent change in O_2 uptake per hour (with reference to glucose control)		
	Glucose control	Dihydrostreptomycin (1000 μg)	Aureomycin (10 μg)	Dihydrostreptomycin (1000 μg) +aureomycin (10 μg)
Hour				
1st	75	+ 7.5	− 5.6	−11.7
2nd	71	+ 7.5	− 8.4	−35.4
3rd	67	+ 9.7	− 8.2	−37.4
4th	64	−15.6	−15.5	−69.8
5th	64	−20.7	−18.2	−69.6
6th	58	−28.6	−18.0	−74.4

Endogenous respiration values were subtracted to give the recorded values.

DISCUSSION

The modes of action of the sulfonamides and the antibiotics on bacteria are imperfectly understood. Also, little is known of the intermediary metabolism of the brucella. Consequently, a sarisfactory formulation of the mechanisms of action of the various drugs and combinations used in this study is not possible.

The inability of sulfadiazine to suppress respiration of "resting" brucella cells is in accord with the experience with other bacteria. The initial stimulation of respiration by streptomycin has been reported for *Salmonella typhosa* (Benham, 1947). It is of interest that the slight stimulatory effect of sulfadiazine was continuous over the observed periods, while with streptomycin and dihydrostreptomycin initial stimulation was followed later by a depressing action. Pretreatment of resting cells with sulfadiazine followed by washing revealed no continuing effect on the cells, while such treatment with dihydrostreptomycin showed a continuing effect after contact with these drugs, and indicated a direct relationship between respiratory inhibition and length of time of action of the drugs on the cells. This relationship of exposure time to inhibitory action was also well shown for aureomycin.

The depressing effect of aureomycin on carbohydrate metabolism was demonstrated for substrates of glucose, trehalose, xylose, fructose, and pyruvate. Inhibition of endogenous respiration also was shown. These effects may represent action on one or more fundamental assimilative processes rather than on enzymes directly concerned in glycolysis.

It is of interest that no difference in the effect of aureomycin was found with an aerobic and a carbon dioxide-sensi-

tive strain of *Br. abortus*. The inability of growth factors to abolish inhibition by aureomycin was to be anticipated.

In the experiments using combinations of aureomycin and dihydrostreptomycin, a synergistic action was shown, the two together exerting an effect far greater than the summation of their actions singly. With the larger amounts of aureomycin this effect was not shown, but when minimal amounts of aureomycin were used, it became readily apparent. The addition of sulfadiazine to aureomycin or to the combination of aureomycin and dihydrostreptomycin lessened the inhibitory action. This effect demonstrated in vitro does not necessarily have any bearing on the clinical use of this triple combination, which has been found effective in the treatment of human brucellosis. The experiments quoted showing the effect of sulfadiazine, streptomycin, and the combination of these two drugs on brucella populations in nutrient mediums establish their synergistic effect in inhibiting multiplication of brucella and in production of eventual death of the populations.

SUMMARY

The effect of sulfadiazine and certain antibiotics on respiration of brucella was studied in the Warburg apparatus. Sulfadiazine did not inhibit respiration with a glucose substrate; streptomycin and dihydrostreptomycin exhibited an early stimulatory effect followed by inhibition. Aureomycin, chloromycetin, and terramycin all produced inhibition, that with aureomycin being most marked. Certain combinations of aureomycin and dihydrostreptomycin (or streptomycin) showed a synergistic inhibitory effect. Aureomycin was also shown to inhibit respiration with trehalose, xylose, fructose, and pyruvate substrates. No difference was noted in the action of aureomycin on an aerobic and a carbon dioxide-sensitive strain of *Brucella abortus*. Cells treated with aureomycin and streptomycin showed continued action of the drugs after washing; those treated with sulfadiazine did not.

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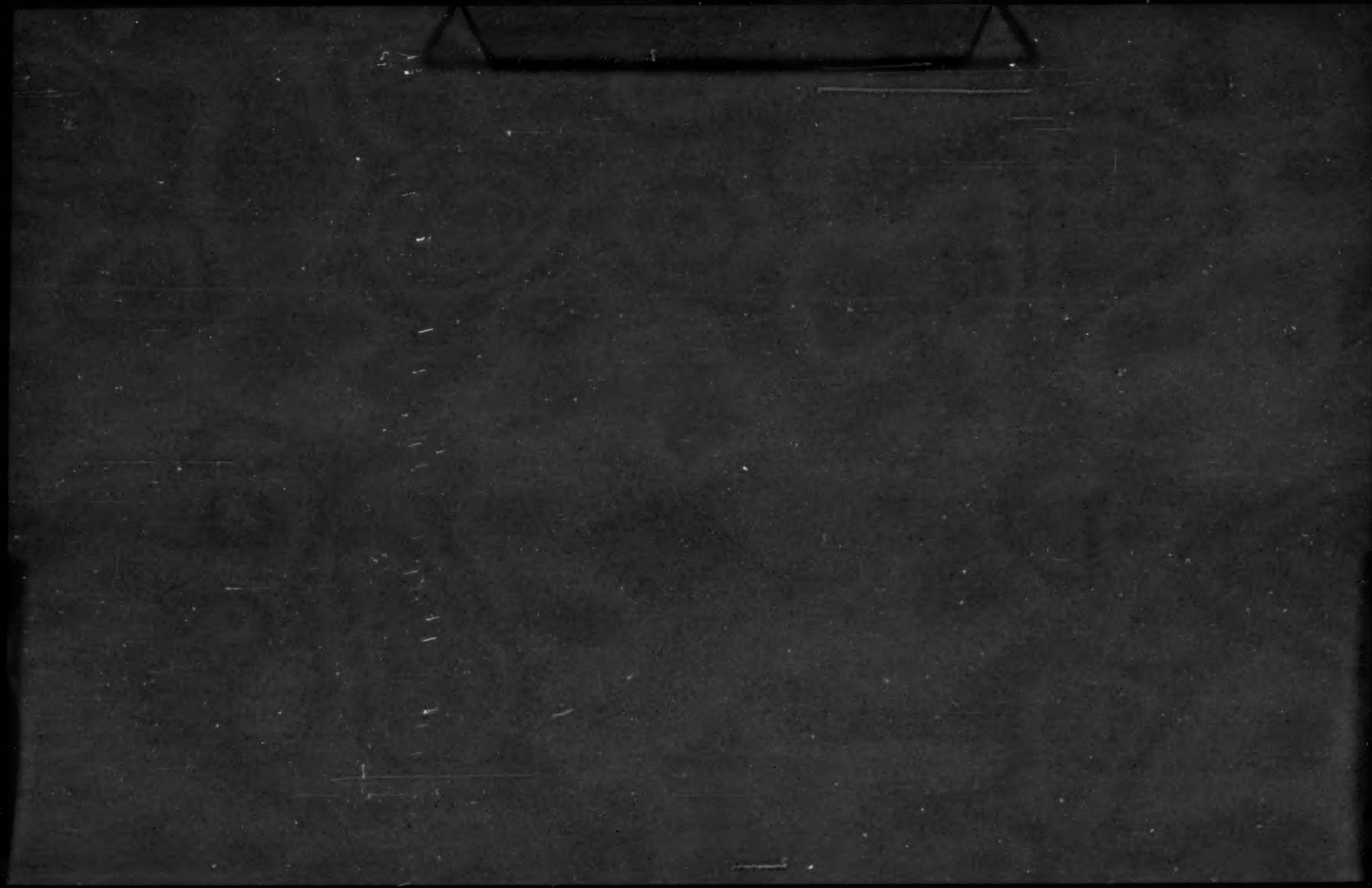

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